

# The eDNA Society

## NEWSLETTER



### contents

- 1 . Greetings from the Representative Director/President of The eDNA Society
- 2 . Report on the 7<sup>th</sup> Annual Meeting of The eDNA Society in Tsukuba
- 3 . Frontiers in Environmental DNA Study (7th)
- 4 . Cutting-Edge Environmental DNA Analysis (4th)
- 5 . Donation Report
- 6 . Newsletter Editorial Team / Editor's Note
- 7 . Board and Committee Members of The eDNA Society

no. **7**

<b>Greetings from the Representative Director/ President of The eDNA Society</b> .....	2
<b>Report on the 7<sup>th</sup> Annual Meeting of The eDNA Society in Tsukuba</b> .....	3
Meeting report.....	3
List of presentation award winners.....	7
Reports on symposium and sessions.....	9
Public symposium: New insights from environmental DNA analysis to control invasive alien species.....	9
Organized session 1: The frontier of environmental DNA analysis: Challenging unresolved problems.....	11
Organized session 2: Frontiers in environmental DNA-based migratory fish studies.....	12
Contributed session 1: New approaches to assessing the environmental impact of chemicals: Bridging ecotoxicology and ecology through environmental DNA/RNA monitoring.....	13
Contributed session 2: Environmental DNA: Frontiers of on-site implementation.....	14
<b>Frontiers in Environmental DNA Study (7th)</b>	
Monitoring fish communities in UNESCO World Heritage natural marine sites: A large-scale UNESCO environmental DNA survey utilizing citizen science.....	15
<b>Cutting-Edge Environmental DNA Analysis (4th)</b>	
A passive environmental DNA sampling method using natural sponge skeletons.....	17
<b>Donation Report</b> .....	20
<b>Newsletter Editorial Team/Editor's Note</b> .....	21
<b>Board and Committee Members of The eDNA Society</b> .....	24

# Greetings from the Representative Director/ President of The eDNA Society

## **Toshifumi MINAMOTO**

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



The past year has seen the rapid acceleration of movement toward the international standardization of eDNA technologies. Some items presently in draft documents for the ISO (International Organization for Standardization) standardization of eDNA methods are being voted on, and discussions on other items have begun within the International eDNA Standardization Task Force (iESTF). The eDNA Society has asked board members to join the teams for each item, and we are continuing discussions to ensure a high international standard and minimal conflict with our existing manual. Participants from each country held equitable discussions within these teams, and I am hopeful that a well-organized ISO document will be produced. This movement will continue for the next 2-3 years, and each item is anticipated to become an official ISO in turn.

In February 2025, I attended the Southern eDNA Society conference in Wellington, New Zealand, where I learned about the progress of international research, particularly in Oceania. I was amazed at the speed of movement and the scope of many large-scale projects currently underway, all of which appear to be implemented through collaborations between industry, government, and academia, in a manner similar to that of to our own society. I also had the opportunity to speak candidly with key members of the Southern eDNA Society, and I believe that we can work together to implement a number of projects in the future.

Many presentations given at the 7<sup>th</sup> Annual Meeting of the eDNA Society held in Tsukuba, Japan, in December 2024 indicated promising new eDNA developments based on exciting ideas. Although I will not describe the findings of each study here, I hope that the members of our society will continue to work toward developing new methods and exchanging information actively on such methods at our annual conference. I hope that the conference will continue to be a source of fresh surprises in eDNA research. We look forward to your continued support.

# Report on the 7<sup>th</sup> Annual Meeting of The eDNA Society in Tsukuba

## Meeting report

**Natsuko KONDO** (Congress Chair; National Institute for Environmental Studies) and **Noriyuki KOIZUMI** (Congress President; National Agriculture and Food Research Organization, NARO)

The 7<sup>th</sup> Conference of The eDNA Society was held in Tsukuba from November 30 to December 4, 2024, with the theme, “More Accessible with Environmental DNA”. Presentations by high school students were given online on November 30, a public symposium was held as a hybrid event on December 1, and the core program was presented onsite at the Tsukuba International Congress Center on December 2 and 3. Committee meetings were held on December 4 in the NARO Shinbashi Workspace, Tokyo. There were 272 participants in the core program from six affiliated countries, notably including China and Korea.



Group photograph taken at the 7<sup>th</sup> Conference of The eDNA Society in Tsukuba.

A wide range of basic to applied research results were shared and discussed daily, including presentations by high school students, a public symposium, two organized sessions, two contributed sessions, and 79 poster presentations (67 by general members and 12 by high school students). The meeting also featured seven commercial exhibitions and two sponsored seminars that attracted large, attentive audiences. The noticeable increase in the number of participants and the lively atmosphere assured us that this conference would continue to grow and thrive in years to come.

The organizing committee adopted three initiatives at this meeting by way of contributing to the future of eDNA research, as outlined below. Reports on the public symposium and various other meetings are provided in other articles within this newsletter.

### **Initiative 1: Online presentations by high school students**

Poster presentations by high school students have been conducted at previous meetings. However, scheduling and venue decisions can significantly restrict the participation of high school students at onsite meetings. Therefore, we held an online presentation session that allowed participation from any location, to facilitate discussion. Each of the 12 presentations were highly ambitious and impressive, and the question/answer sessions were lively and engaging. Through the invaluable cooperation of five judges, we awarded a Grand Prize and an Excellence Award. In addition to providing a valuable opportunity for high school students to present and discuss their work, this event offered highly stimulating ideas and discussions for the general participants. Despite the additional effort required on the part of the organizing committee to maintain this online session, we believe that it provided valuable opportunities for all involved and hope that it will continue to be part of the annual meeting.



Poster presentation session.

### **Initiative 2: Plenary sessions**

At the 7<sup>th</sup> Annual Meeting, we adopted a plenary format for all oral sessions, meaning that all participants attended a single presentation together. In recent meetings, multiple contributed sessions have been held concurrently. However, both participants and organizers have requested to be able to participate in all sessions. Overall, the plenary format appeared to be well received. Although all sessions of the annual meeting share the common keyword “eDNA”, the research approaches and objectives can vary widely among sessions. While the venue and

schedule may impose constraints in many years, we believe that the occasional adoption of a plenary format for contributed sessions could revitalize the annual meeting.



Oral presentation session.

### Initiative 3: Coordinated poster designs

For the Tsukuba meeting, matching posters were created to raise awareness of The eDNA Society among a broader audience. A yellow poster was designed to advertise the public symposium (below, left), and a green poster was designed to advertise the entire meeting (below, right). Both posters featured the DNA double helix, representing the detection of organisms through eDNA. The public symposium poster featured the theme of invasive species, whereas the general meeting poster showed species native to Ibaraki Prefecture. We believe that the strongly coordinated design of these posters may have contributed to the greater numbers of non-members and same-day participants at this meeting compared to previous years, although their actual impact is uncertain.



Coordinated meeting posters.

## Remaining issues

The response to internationalization was one of the most important challenges for the 7<sup>th</sup> Annual Meeting. The eDNA Society includes many members from Japanese companies and local government research institutes, creating a need for efficient information gathering and discussion in Japanese. How can we maintain a platform for collaboration between industry, academia, and government in Japan while deepening discussions among international and diverse participants? Inevitably, there must be a tradeoff. In this annual meeting, oral presentations were given primarily in Japanese, and participants who did not speak Japanese were able to connect via Zoom, using its automatic translation feature. Although we received positive comments about the automatic translation, we believe that it also caused frustration among those who did not speak Japanese. The language used at the annual meeting and the issue of internationalization are important issues that will impact the future of The eDNA Society. Therefore, it will be necessary to clarify further the strategic direction of our society and organize our meetings accordingly.

## Final remarks

In his closing remarks, Dr. Minamoto, the President of The eDNA Society, stated that, “eDNA analysis is an evolving technology, but we see it as something that will be further developed through practical use and collective discussion.” We frequently hear about eDNA at various scientific conferences other than The eDNA Society annual meeting. The role of our society is becoming increasingly significant, because eDNA analysis technology is widely utilized in various comprehensive research studies. We look forward to learning of new developments at the next annual meeting, at Yamaguchi.

Finally, on behalf of the organizing committee, we extend our gratitude to all participants of this year's meeting, the sponsors and exhibitors, and those who judged posters and online presentations by high school students, as well as to the Tokyo branch of the Ecology and Civil Engineering Society for providing equipment for online and hybrid sessions.

## Poster Awards

### Best General Poster Award (Basic Science)

**P-56 Detection of high-resolution temporal variation patterns in fish populations and communities through environmental DNA metabarcoding surveys**

Keisuke Ota<sup>1</sup>, Shota Suzuki<sup>2</sup>, Takuzo Abe<sup>2</sup>, Akihiro Dazai<sup>3</sup>, Gen Iwashita<sup>1</sup>, Gohki Kasahara<sup>1</sup>, Hiroto Oikawa<sup>2</sup>, Kouhei Ohmuro<sup>1</sup>, Mayu Suzuki<sup>1</sup>, Minoru Kasada<sup>4</sup>, Naoma Motomatsu<sup>1</sup>, Syogo Kobayashi<sup>3</sup>, Tatsuya Miyamoto<sup>1</sup>, Michio Kondoh<sup>1</sup>

1. Tohoku University, 2. Minamisanriku Nature Center,  
3. Center for Sustainable Society, 4. Hokkaido University

### Best General Poster Award (Applied Science)

**P-58 Calculation of diatom pollution indicator values for aquatic ecosystem health assessment based on biofilm eDNA.**

Keonhee Kim<sup>1</sup>, Kyu-Jin Kim<sup>2</sup>, Hyeonjin Cho<sup>3</sup>, Jeong-eun Na<sup>4</sup>, Min-Ho Jang<sup>2</sup>

1. Konkuk University, 2. Kongju National University, 3. Encounter the ecology Co.,  
4. Chonnam National University

### Outstanding General Poster Award (Basic Science)

**P-43 Elucidating the spatiotemporal distribution dynamics of black bass species in Lake Biwa**

Atsushi Okada<sup>1</sup>, Kei Wakimura<sup>2</sup>, Kimiko Uchii<sup>2</sup>, Qianqian Wu<sup>1</sup>, Toshifumi Minamoto<sup>1</sup>

1. Kobe University, 2. Osaka Ohtani University

**P-34 Exploring fish biodiversity in Urauchi River mangroves: An eDNA metabarcoding study of longest river in Okinawa, Japan**

Bernadeth Grace Suerte Pananganan<sup>1,2</sup>, Yukinobu Isowa<sup>2</sup>, Maria Daniela Artigas Ramirez<sup>2</sup>, Tadashi Kajita<sup>1,2</sup>

1. UGSAS Kagoshima University, 2. TBRC University of the Ryukyus

### Outstanding General Poster Award (Applied Science)

**P-48 Estimation of distribution factors of *Opisthorchis viverrini*, in Savannakhet province, Lao PDR**

Riko Matsuo<sup>1</sup>, Phoyphaylinh Prasayasith<sup>2</sup>, Joseph Evangelista Valencia<sup>2</sup>,

Mark June Valiente Revolteado<sup>2</sup>, Poom Adisakwattana<sup>3</sup>, Tippayarat Yoonuan<sup>3</sup>,

Orawan Phuphisut<sup>3</sup>, Yanin Limpanont<sup>3</sup>, Marcello Otake Sato<sup>4</sup>, Megumi Sato<sup>2</sup>,  
Tiengkham Pongvongs<sup>5</sup>, Toshifumi Minamoto<sup>1</sup>

1. Kobe University, 2. Niigata University., 3. Mahidol University, 4. Nupmls,  
5. Savannakhet Provincial Health Department

## High School Student Presentation Awards

### Best Presentation Award

**PS-03 Fish community structure across the Yura River system, Kyoto,  
revealed by environmental DNA**

Kyoto Prefectural Fukuchiyama High School

Mayu Shiomi, Manaka Yura, Yoshiie Tanaka, Takeaki Aratani, Richika Suzuki,  
Junta Fujita

### Outstanding Presentation Award

**PS-05 Investigation of the fish fauna of rivers in the Noto area using  
environmental DNA**

Ishikawa Prefectural Nanao High School

Harune Asada, Nene Kanazawa, Sho Takezawa, Shun Tanaka, Takaaki Nobuta,  
Iroha Yamaguchi



Presentation award winners.

### ■ Public symposium: New insights from environmental DNA analysis to control invasive alien species

**Noriyuki KOIZUMI** (Congress President, 7<sup>th</sup> Annual Meeting of The eDNA Society; NARO)

The public symposium of the 7<sup>th</sup> Annual Meeting of The eDNA Society was held on December 1, 2024, at the Leo Esaki Main Convention Hall of the Tsukuba International Congress Center, with a total of 395 participants (121 in person and 274 online). Co-hosted by NARO and The eDNA Society, the symposium focused on the monitoring and management of invasive alien species using eDNA technology.

This symposium was organized as a platform to share the outcomes of a research project commissioned by the Ministry of Agriculture, Forestry and Fisheries: “Development of Management Technologies for Invasive Alien Species Causing Agricultural Damage.” This event introduced the latest research on eDNA technology and aimed to promote collaboration between researchers, government agencies, and private companies, to further advance and implement this technology in society.

In his opening remarks, Dr. Shori Yamamoto (NARO) emphasized the importance of eDNA technology in invasive species management. Next, a keynote lecture by Dr. Koichi Goka (National Institute for Environmental Studies) presented case studies that included work on the chytrid fungus (*Batrachochytrium dendrobatidis*), mongooses, invasive ants, and the Asian hornet (*Vespa velutina*), demonstrating how eDNA can be used for effective early detection, distribution mapping, and the evaluation of control measures.



Public symposium on the use of eDNA in controlling invasive alien species.

Subsequent presentations introduced practical applications of eDNA technology. Dr. Kenji Ito (NARO) reported on countermeasures against water flow obstruction caused by golden mussels (*Limnoperna fortunei*) in the Kasumigaura River and Naka River systems, demonstrating that early detection via eDNA contributes significantly to agricultural canal management. Mr. Masatoshi Nakamura (Idea Consultants, Inc.) described the development and application of mollusk metabarcoding technology, highlighting an impact assessment of reservoir water level manipulation for the removal of invasive bivalves from native fish and mollusk communities. Dr. Chinatsu Kozakai (NARO) delivered a presentation on the application of eDNA in wildlife damage management, showcasing new uses for eDNA in the forensic analysis of feeding traces and individual age estimation. She also indicated that further research is needed to extend the application of eDNA technology to terrestrial organisms.

A panel discussion chaired by Mrs. Keiko Muraoka (Public Works Research Institute) covered the standardization of eDNA technology and database development, the publication and risk management of invasive species detection data, the utilization of biodiversity data for rare species conservation, and policy implementation in collaboration with government agencies. These discussions particularly emphasized the necessity of expanding reference DNA sequences and data sharing, to enhance the effectiveness of eDNA technology. Balancing the advantages and risks of disclosing detection results was identified as a key challenge in ensuring appropriate management. The establishment of inter-ministerial roundtables and regular policy discussions was also identified as an important step forward.

Participants raised various questions, including the applicability of eDNA technology for monitoring terrestrial plants, the risks and management of invasive species data disclosure, and participation in citizen science initiatives. Furthermore, this symposium implemented simultaneous English translation, registration for continued professional development, and childcare services, ensuring an inclusive environment for discussion.

In his closing remarks, Dr. Noriyuki Koizumi (NARO) reflected on the key outcomes of the symposium and expressed his expectations for the further development and social implementation of eDNA technology. Moving forward, expanding efforts in data standardization; strengthening collaboration between government agencies, research institutions, and private companies; and promoting citizen science initiatives will be essential for the broader adoption of eDNA technology.

## ■ Organized session 1 : The frontier of environmental DNA analysis: Challenging unresolved problems

**Masayuki K. SAKATA** (Assistant Professor, Research Faculty of Agriculture, Hokkaido University)

This organized session addressed unresolved issues in eDNA research and presented cutting-edge approaches for tackling these challenges. The first presentation, given by Dr. Masayuki Sakata (Hokkaido University), focused on the detection of hybridization using eDNA. Traditional eDNA analysis has faced challenges in distinguishing the sympatric distribution of parental species and the presence of hybrid individuals. To overcome this limitation, a novel method utilizing digital polymerase chain reaction (PCR) at the single-cell level was developed and validated. The second presentation, by Mr. Itsuki Hirayama (Kobe University), explored the application of eDNA in epigenetics. Conventional eDNA analysis has struggled to infer the physiological states of organisms; the use of DNA methylation was proposed as a potential solution to this issue. Additional discussions raised the possibility of age estimation and detection of spawning-specific eDNA by leveraging methylation information. The third presentation, delivered by Dr. Hiroki Yamanaka (Ryukoku University), examined the impact of PCR bias in eDNA metabarcoding. The results demonstrated that mismatches between primers and template DNA introduce bias, leading to decreased read numbers and potential non-detection of target species. To mitigate this bias, the effectiveness of droplet PCR was highlighted as a promising approach. The fourth presentation, given by Dr. Keiichi Fukaya (National Institute for Environmental Studies), addressed incomplete detection caused by false negatives in eDNA metabarcoding. To account for this issue, species distribution was evaluated by incorporating detection probabilities using an occupancy model. Furthermore, the importance of replication at various stages of the eDNA metabarcoding workflow was emphasized as a means to improve detection accuracy. Both well-recognized unresolved issues and potential emerging challenges in eDNA research were addressed throughout this organized session; the research findings highlighted how novel approaches and alternative technologies are advancing solutions to these challenges. Through such insights, this session constituted a catalyst for enhancing our understanding of the current frontiers of eDNA research and fostering greater interest in unresolved issues, with the expectation of promoting further research and technological advancements in the field.

## ■ Organized session 2: Frontiers in environmental DNA-based migratory fish studies

**Tetsu YATSUYANAGI** (Program-specific Assistant Professor, Field Science Education and Research Center, Kyoto University)

Migratory fish, which change their habitats depending on their life stages, exhibit various migration patterns. Traditional survey methods for tracking their ecology throughout their life histories have required significant time and effort. However, the advent of eDNA methods has offered novel, advantageous approaches for migratory fish studies. This session showcased cutting-edge eDNA research on migratory fish across various environments, discussed its advantages and challenges, and explored future directions. The first speaker, Dr. Tetsu Yatsuyanagi, presented a literature review that highlighted current trends in environmental DNA-based migratory fish research. The second speaker, Dr. Kimiko Uchii (Osaka Ohtani University), presented a novel conservation study on Honmoroko (*Gnathopogon caerulescens*), a fish endemic to Lake Biwa, focusing on its genetic diversity and natal homing behavior using eDNA haplotyping. The third speaker, Yusuke Kumai (The University of Tokyo), presented a study on factors determining migratory species composition on Yakushima Island, which hosts various diadromous fish, using eDNA metabarcoding. The fourth speaker, Dr. Hiroaki Murakami (Tohoku University), presented research estimating the seasonal distribution of juvenile and adult Japanese sea bass (*Lateolabrax japonicus*) by analyzing eDNA concentrations in relation to salinity and water temperature. Each presentation stimulated active discussions on ecological perspectives. Finally, the co-organizer, Dr. Hitoshi Araki (Hokkaido University), gave a talk based on his salmonid research, outlining the current challenges and future prospects of eDNA methods. A general discussion followed, focused mainly on technological constraints and potential solutions. While eDNA is highly effective for capturing the spatiotemporal distribution of migratory fish, a well-planned survey design that accounts for the target life stages and environmental conditions is essential. We hope that this session will contribute to advancing the application of eDNA for the effective investigation and monitoring of migratory fish, including commercially and ecologically valuable species.

## ■ Contributed session 1 : New approaches to assessing the environmental impact of chemicals: Bridging ecotoxicology and ecology through environmental DNA/RNA monitoring

**Kyoshiro HIKI** (National Institute for Environmental Studies),

**Hiroshi HONDA** (Kao Corp.)

In his opening remarks, Dr. Hiroshi Honda (Kao Corp.), one of the organizers of this contributed session, explained that chemical pollution and biodiversity loss pose serious threats to sustainability and outlined the highly anticipated potential of environmental DNA and RNA in techniques for monitoring biota and biological stresses. The second speaker, Dr. Takashi Nagai (NARO), presented a talk entitled “Application of environmental DNA techniques to the ecological assessment of pesticides.” He introduced the use of eDNA metabarcoding to assess the ecological impacts of pesticides on aquatic insects and diatoms. The third speaker, Dr. Noriko Uchida (Tohoku University), delivered a presentation entitled “Can changes in benthic macroinvertebrate communities be detected in metal-contaminated rivers using environmental DNA?” She shared eDNA metabarcoding results for benthic animals sampled from both contaminated and uncontaminated rivers near mining areas. The fourth speaker, Dr. Yasuaki Inoue (Kao Corp.), presented “Ecological effect assessment linking ecotoxicology and ecology using environmental RNA metabarcoding.” He reported that, for capturing changes in community composition, environmental RNA metabarcoding is more sensitive than eDNA in rivers contaminated with surfactants. Dr. Kaede Miyata (Kao Corp.) introduced a novel method in her presentation, “Fish environmental RNA sequencing sensitively captures accumulative stress responses through short-term aquarium sampling.” Using medaka exposed to surfactants as a case study, she demonstrated how environmental RNA sequencing can be employed to evaluate the stress responses of aquatic organisms. Finally, Dr. Kyoshiro Hiki (National Institute for Environmental Studies), another organizer of this session, presented “Development of environmental RNA analysis technologies in laboratory toxicity testing and its application to field.” He proposed several strategies for efficiently performing eRNA sequencing on macroorganisms in field settings with abundant microbial RNA.

In summary, the contributed session featured a diverse range of speakers from industry, academia, and national research institutes who shared various research case studies that utilized environmental DNA/RNA. These efforts could contribute to a multifaceted visualization of the impacts of chemical pollution and aid in verifying the effectiveness of nature-positive strategies. Although ecotoxicology has often been confined to laboratory experiments, it may newly evolve through integration with ecological biodiversity assessments such as environmental DNA/RNA analyses. We hope to continue strengthening our collaborative relationships with various academic societies, including The eDNA Society, and to share our

research findings broadly with society.

## ■ Contributed session 2: Environmental DNA: Frontiers of on-site implementation

**Mika GUNJI** (Nippon Koei Co., Ltd.), **Jiro OKITSU** (OYO Co., Ltd.),  
**Kenji TSURI** (KenKan Consultants Co., Ltd.), **Munehiro OOTA** (KenKan Consultants Co., Ltd.), **Keiko MURAOKA** (Public Works Research Institute, PWRI)

In this contributed session, a number of construction consulting companies gathered to discuss issues and case studies related to eDNA. Ms. Mika Gunji (Nippon Koei Co., Ltd.) described a case study involving visual and eDNA surveys of amphibians in a mountain stream, and reported on database shortages for species other than fish. Mr. Jiro Okitsu (OYO Co., Ltd.) introduced a case study conducted at the Miharu Dam, including findings on the optimal timing of water sampling for fish species detection and a comparison between the amount of fish present and the quantity of metabarcoding analysis data. Mr. Hajime Inoue (CTI Engineering Co., Ltd.) presented a case study that estimated the spawning time of cherry salmon at the Uryu River Dam using the concentration ratio of nuclear/mitochondrial eDNA. Dr. Ryota Yokoyama (KenKan Consultants Co., Ltd.) introduced a case study that showed a rough consistency between eDNA and diving survey data for fish in an erosion-controlled stream. Finally, Ms. Keiko Muraoka (PWRI) described the efforts of the PWRI to introduce eDNA surveys in censuses of riparian areas from 2026.

Dr. Keigo Nakamura (PWRI) moderated the general discussion, and Dr. Toshifumi Minamoto (Kobe University) and Mr. Kazuho Agawa (Ministry of Land, Infrastructure and Transport) participated in a discussion of the practical issues and expectations for future applications of eDNA. Each company faces problems such as database shortages for species other than fish and the need for standard numbers of reads and/or copies. One focus of this contributed session was the necessity for accumulating basic information by expanding databases and incorporating the latest research on methylation. This contributed session improved our understanding of practical applications of eDNA and identified key issues that remain to be resolved. We hope that industry, government, and academia will continue to collaborate in future research activities.

# Frontiers in Environmental DNA Study (7th)

## Monitoring fish communities in UNESCO World Heritage natural marine sites: A large-scale UNESCO environmental DNA survey utilizing citizen science

**Masaki MIYA** (Research Organization for Nano & Life Innovation, Waseda University)

From September 2022 to July 2023, the United Nations Educational, Scientific and Cultural Organization (UNESCO) spearheaded an innovative research initiative that integrated citizen science with eDNA methodologies to evaluate the effects of climate change on marine ecosystems. This project, entitled “UNESCO eDNA Expeditions” (<https://www.unesco.org/en/edna-expeditions>), targeted 21 of the 51 UNESCO World Heritage natural marine sites. The primary aim of the project was to monitor current biodiversity, with a specific focus on fish communities, through the active participation of local citizens.

The author contributed to the project as a member of the advisory board, engaging from the earliest planning phases. Critical topics that were discussed with fellow board members encompassed the design of sampling kits distributed to participants, the volume of water to be filtered, guidelines for sample collection and filtration, protocols for eDNA preservation, the selection of primers for metabarcoding (simultaneous detection of multiple species), and strategies for analyzing and interpreting the extensive sequencing data generated by high-throughput sequencing technologies.

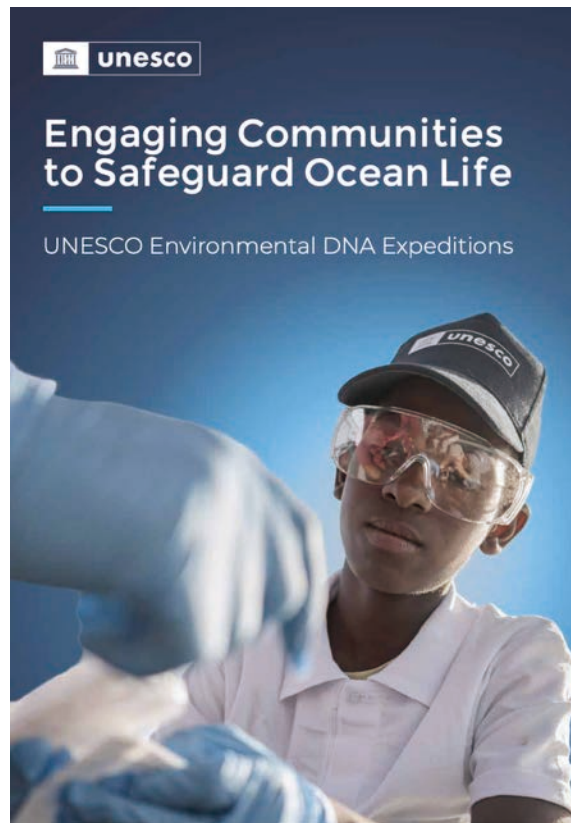
This initiative mobilized over 250 citizen scientists from 19 countries, resulting in the detection of more than 4,000 marine species, with fish constituting over half of the identified taxa. Significantly, 120 species listed on the International Union for Conservation of Nature Red List of Threatened Species were recorded, underscoring the susceptibility of these organisms to habitat shifts precipitated by rising ocean temperatures. A notable example is the white-spotted guitarfish (*Rhynchobatus australiae*), which was detected in several Australian marine regions, exemplifying species vulnerable to climate-induced changes.

The survey demonstrated that a mere 1.5-L seawater sample from a single site could yield DNA from several hundred species. This approach enabled the acquisition of biodiversity data within hours, a process that would traditionally span years using conventional diving or gear-based collection methods. The simplicity of the sampling technique allowed participation by individuals as young as six years old, incorporating an educational dimension that fosters public engagement in marine conservation.

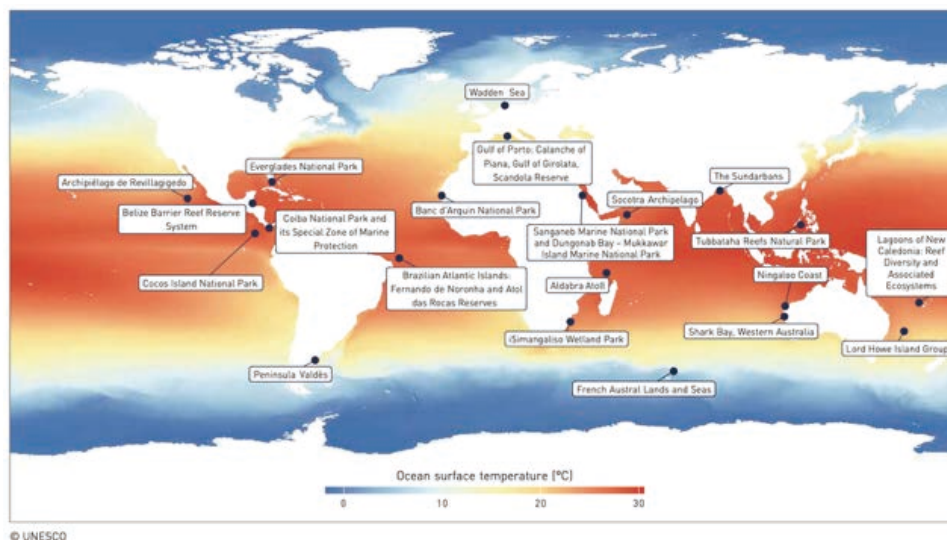
This project is the first global endeavor to merge citizen science with

advanced eDNA technology on such an expansive scale. Furthermore, UNESCO has significantly advanced the principles of open science by ensuring that all collected data and protocols are freely accessible. This commitment to transparency empowers researchers and policymakers worldwide to harness data for the formulation of robust, evidence-based conservation strategies.

Preliminary findings from this initiative are detailed in a UNESCO report entitled “Engaging Communities to Safeguard Ocean Life: UNESCO Environmental DNA Expeditions”, which is freely available to download (<https://www.doi.org/10.58337/CBXU3518>). Comprehensive analytical results are anticipated to be published in peer-reviewed journals.



Cover of the United Nations Educational, Scientific and Cultural Organization (UNESCO) report on the “UNESCO eDNA Expeditions” project.



Locations of the 21 UNESCO World Heritage marine sites participating in the UNESCO eDNA expedition.

# Cutting-Edge Environmental DNA Analysis (4th)

## A passive environmental DNA sampling method using natural sponge skeletons

**Ryohei NAKAO** (Graduate School of Science and Technology for Innovation, Yamaguchi University)

Sampling for environmental DNA (eDNA) analyses typically relies on the direct collection of environmental samples from water, soil, or air. Water samples are most frequently used in eDNA analyses because aquatic organisms are often the target species. However, a simple, labor-saving passive sampling method was recently proposed, in which a material that can trap eDNA is placed in the environment and collected after a given period. Passive eDNA sampling has high potential for obtaining time-cumulative biological information on long-term environmental exposure. Various eDNA-trapping materials have been used in previous eDNA studies, including natural living sponges<sup>1</sup>, activated carbon, clay<sup>2</sup>, and calcium compounds<sup>3</sup>. Our research group at Yamaguchi University developed a passive sampling method that uses relatively inexpensive natural sponge skeletons composed of spongin fibers to capture eDNA for river and marine environmental biomonitoring, in collaboration with Nippon Koei Co., Ltd.

In a recent case study examining fish fauna at five sites along a river in Japan, sponge skeletons 1–2 cm in diameter were strapped to weighted baskets and left in the water for a period ranging from overnight to 24 h (Fig. 1). Then, the sponge

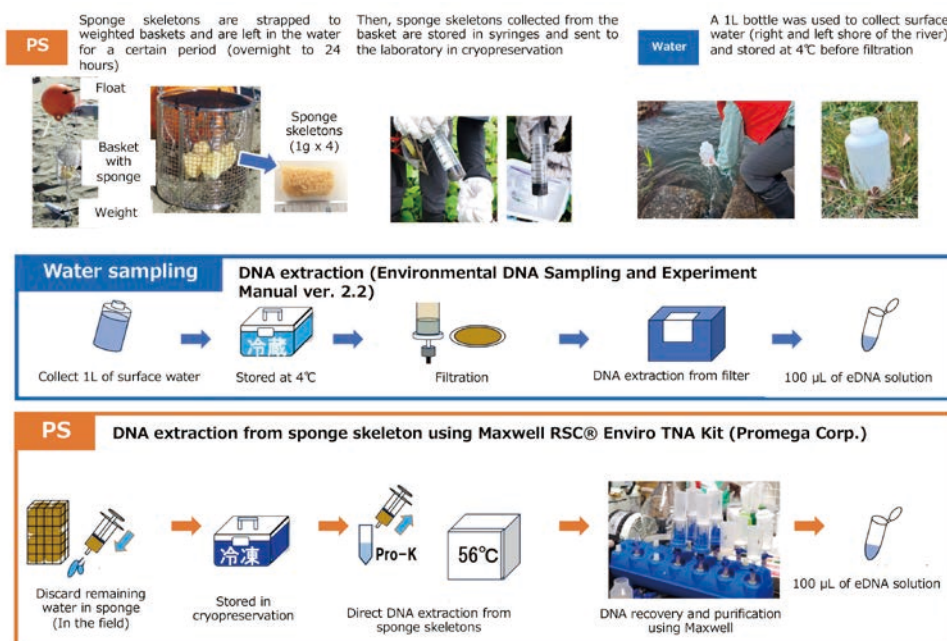


Fig. 1 Illustrations of two eDNA sampling methods: (upper) a traditional water filtration method and (lower) a passive method based on eDNA capture by sponge skeletons.

skeletons were collected from the baskets, stored in the syringe, cryopreserved, and sent to the laboratory. DNA was extracted from the sponge skeletons using a Maxwell RSC Enviro TNA Kit DNA Purification kit (Promega, Madison, WI, USA), with slight modifications to improve DNA recovery. For comparison, we analyzed data obtained using our passive sampling technique and from filtered river water samples based on fish eDNA metabarcoding (MiFish) primers.

The results showed that passive sampling yielded a higher number of species than the filtration method at all sites (Fig. 2). The largest difference in the number of species detected between the two methods was observed at the river mouth, where many marine fishes inhabiting the coastal area were detected only through passive sampling. Nocturnal fish were detected in more sites under passive sampling than water filtration. Thus, passive sampling was more effective for the collection of eDNA from coastal fish, which occasionally enter the river mouth, and from nocturnal fish, which are not active during the day. These findings suggest that passive sampling using sponge skeletons as an eDNA-trapping material is suitable for time-cumulative eDNA monitoring. Optimizing the proposed passive sampling method remains challenging, and factors such as the number of sponges and immersion time, as well as applications to other taxa or in environments other than water will require further study. The sponge skeleton methodology could also be made more user-friendly.

We thank Y. Akamatsu, M. Inaba, F. Imamura, M. Gunji and many others from Yamaguchi University and Nippon Koei Co. Ltd. for their cooperation in laboratory work and field surveys throughout the development of our passive sampling method. We also thank J. Fujiwara and Y. Yasue from Promega for providing technical support during DNA extraction and purification.

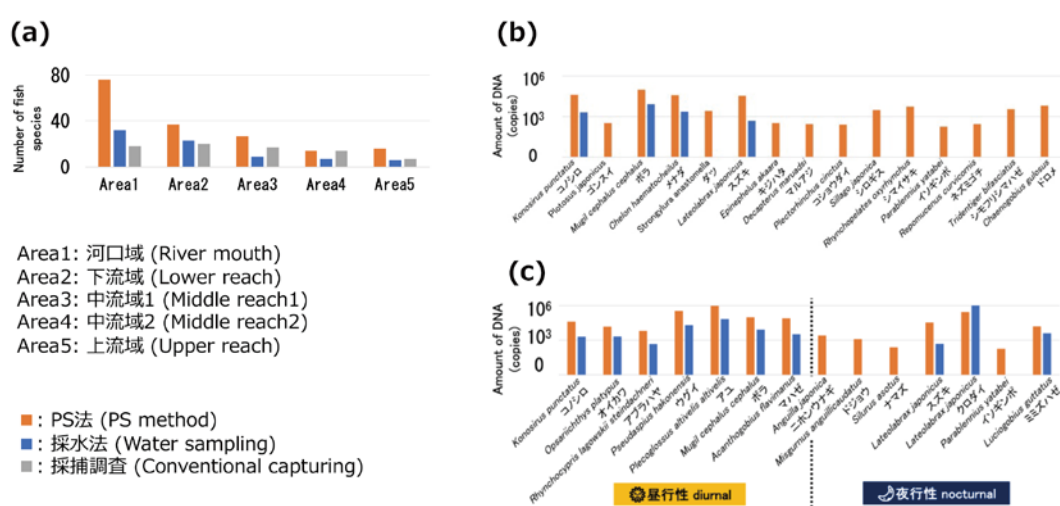


Fig. 2 Fish fauna detection results obtained at five river eDNA sampling sites using three fish monitoring methods: passive sampling based on eDNA capture by sponge skeletons, water sampling and filtration, and a conventional capture-based survey. (a) Differences in the number of detected species among the three methods. (b) Comparison of eDNA concentrations for shore fishes at site 1 between the passive sampling and water filtration methods. (c) Comparison of eDNA concentrations for diurnal and nocturnal fishes between the passive sampling and water filtration methods.

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# Donation Report

The eDNA Society received a generous donation in 2024, for which it is sincerely grateful:

**Hiyoshi Ecological Services** (Omihachiman, Shiga)

220,000 JPY

# Newsletter Editorial Team/Editor's Note

## Newsletter Editorial Team

**Editor-in-Chief** **Yuki MINEGISHI** (Associate Professor, Atmosphere and Ocean Research Institute, The University of Tokyo)

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**Aya TAKEUCHI** (Assistant Professor, Faculty of Agriculture, Kindai University)

**Tetsu YATSUYANAGI** (Program-specific Assistant Professor, Field Science Education and Research Center, Kyoto University)

Three new members of the public relations committee were introduced. Short *curricula vitae* (CVs) for these members are provided below.

### Yuki MINEGISHI

#### Short CV:

Ph.D. in Agriculture from The University of Tokyo, followed by postdoctoral and other positions at Université Montpellier II, Leiden University, etc. Current position held since 2021.

#### Message:

Environmental DNA techniques have greatly advanced within the last several years, and will broaden research horizons in the fields of ecology and behavioral science. I hope to contribute to the exciting research conducted by society members; my work will address eDNA frontiers through international expansion and collaboration among new research by community members.



## Aya TAKEUCHI

### Short CV:

Ph.D. in Bioresource Sciences from Nihon University in 2020, Japan Society for the Promotion of Science Research Fellowship for Young Scientists at the University of Tokyo (2020-2023). Current position held since April 2023. Member of The eDNA Society, Japanese Society of Fisheries Science, Ichthyological Society of Japan, and East Asia Eel Society.



### Message:

My research career was inspired by an interest in the spawning behaviors of Japanese eels. Although my knowledge of fish is still shallow, I greatly enjoy many aspects of this research including fish collection, experiments, and manuscript writing. I was recently attracted to the field of eDNA research because it is based on water sampling. I aim to further expand the practical applications of eDNA in ecology.

## Tetsu YATSUYANAGI

### Short CV:

Ph.D. in Agriculture from Hokkaido University (2024). Current position at Maizuru Fisheries Research Station, Kyoto University. Involved in ecological research on fish using eDNA since undergraduate years. Member of The eDNA Society and Ecological Society of Japan.



### Message:

Having left northern Japan where I lived for many years, I now study fish in northern Kyoto. Although I have worked with eDNA for approximately 8 years, I still consider myself a newcomer as a scientist. I have read the newsletters since its first issue, and feel deeply honored to join its editorial team. I will strive to successfully perform my duties as a new member.

**Yuki MINEGISHI** (Editor-in-Chief, The eDNA Society Newsletter)

I have taken over from Kimiko Uchii (Osaka Ohtani University) as editor-in-chief of The eDNA Society newsletter. The public relations committee has two new members, Aya Takeuchi (Kindai University) and Tetsu Yatsuyanagi (Kyoto University); we all hope to fulfill these roles well.

Both The eDNA Society and eDNA techniques have developed rapidly and dramatically in recent years. Internationalization is among the most notable changes to have occurred; broad-ranging expansion involving collaboration between industry, academia, and government, as well as participatory science have focused on eDNA applications that are nature-positive and/or benefit society. These changes were discussed in depth among society members in the annual meeting held in Tuskuba in 2024. The eDNA Society aims to advance both academic research and its involvement in society, as two wheels connected by the shaft of eDNA technologies. The public relations committee will strive to make this newsletter a platform for the exchange of valuable information related to eDNA. If you would like to contribute information to this newsletter, please contact the public relations committee members. Finally, I express my deepest gratitude to the authors who contributed to this newsletter.

I look forward to seeing you all at the next annual meeting in Yamaguchi in December 2025.

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Editor-in-Chief: Yuki MINEGISHI

The eDNA Society Office (c/o Ryukoku University)

1-5 Yokotani, Seta Oe-cho, Otsu, 520-2194 Japan

email [office@ednasociety.org](mailto:office@ednasociety.org)

website <https://ednasociety.org/en>