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社会インフラとしての
環境DNAの未来を描く

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要旨集

Abstracts



【P-01】

ANEMONE：自然情報社会を支える情報基盤の構築

— 標準化された生物多様性観測から意思決定へ —

ANEMONE: Towards a Nature Information Infrastructure for Decision-Making

– *Building standardized biodiversity observations to support society*

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環境 DNA は、生物多様性を観測する革新的な技術として発展してきた。一方で近年、その役割は研究利用にとどまらず、行政、企業、地域社会、市民科学など、多様な主体の意思決定を支える「自然情報」へと拡大しつつある。本発表では、全国規模の環境 DNA 観測ネットワーク ANEMONE のこれまでの取組と今後の展望を紹介する。ANEMONE では、標準化された観測プロトコル、品質保証、データ共有基盤、企業・行政・市民との連携を通じて、生物多様性観測を社会の情報基盤へと発展させることを目指している。さらに、ANEMONE Global、ANEMONE Biz、BRIDGE プロジェクト等の取組を踏まえ、観測から自然情報、意思決定、社会実装へとつながる「自然情報社会」の実現に向けたビジョンを提案する。

Environmental DNA (eDNA) has revolutionized biodiversity monitoring by enabling efficient, non-invasive, and standardized observations across diverse ecosystems. More recently, however, its role has expanded beyond scientific research toward supporting decision-making by governments, industries, local communities, and citizens through the provision of reliable biodiversity information.

This poster presents the ANEMONE initiative, a nationwide biodiversity observation network in Japan, and discusses its vision for developing a Nature Information Infrastructure. ANEMONE integrates standardized observation protocols, quality assurance, data-sharing systems, and collaborations among researchers, government agencies, private companies, and citizen scientists to establish a robust foundation for biodiversity information.

Recent developments, including ANEMONE Global, ANEMONE Biz, and the BRIDGE program, demonstrate how standardized biodiversity observations can support environmental management, business applications, and public policy. These initiatives illustrate the transition from biodiversity observation as a scientific activity to biodiversity information as societal infrastructure.

We propose that the next generation of biodiversity observation should focus not only on data collection but also on generating decision-ready biodiversity information that can support evidence-based decision-making across society. ANEMONE provides a practical framework for this transition by linking standardized observations with information sharing, quality assurance, and cross-sector collaboration.

This poster presents ANEMONE as a model for building a Nature Information Infrastructure that supports a future Nature Information Society.

【P-02】

市民参加型の環境 DNA 調査を支援するデータ収集・成果共有基盤の構築

Development of an Information Infrastructure for Data Collection and Knowledge Sharing in Participatory Environmental DNA Surveys

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環境 DNA (eDNA) 解析による生物多様性の広域・長期モニタリングには、多様な地域での試料採取と地域環境情報の蓄積が必要であり、市民の地域固有の知見を活かす市民参加型研究 (Participatory Science) との親和性が高い。一方で、多数の市民が関わるコミュニティ型調査では、調査記録の入力・転記エラーへの対応と、研究成果を分かりやすく共有する仕組みの整備が課題となる。本発表では、アースウォッチ・ジャパンの「環境 DNA を用いた魚類調査プロジェクト」における実践を通じて、データ入力ツール eFieldNote と成果共有基盤 UODAS を紹介する。eFieldNote は調査地点メタデータの標準化と記録品質の向上を支援し、UODAS は eDNA 解析結果を GIS と連携して共有・可視化する。大会では、運用から得た知見と課題を共有し、市民参加型環境 DNA 調査を支える情報基盤のあり方を議論したい。

Environmental DNA (eDNA) analysis has become an effective approach for large-scale and long-term biodiversity monitoring. Because successful eDNA monitoring relies on sustained sampling across diverse regions together with contextual environmental information, it is particularly well suited to participatory science that draws upon the local knowledge of community participants. However, community-based surveys involving large numbers of participants face challenges in standardizing metadata associated with sampling sites, reducing data entry and transcription errors, and establishing effective mechanisms for sharing research outcomes while promoting mutual knowledge sharing among participants.

In this presentation, we report the design, development, and operation of eFieldNote, a field data collection tool, and UODAS, a knowledge-sharing platform, through their application in the Earthwatch Japan Fish Monitoring Project using Environmental DNA. eFieldNote is a mobile application that enables flexible creation and management of digital survey forms, standardizes the collection of sampling-site metadata, and improves data quality by reducing data entry and transcription errors. UODAS is an HTML-based interactive platform for sharing eDNA analysis results, enabling comparisons of fish community composition among sampling sites, rankings of detected taxa, and integrated visualization with GIS. These functions support researchers and community participants in sharing survey outcomes and promoting mutual understanding.

At the conference, we will share practical experience gained through the implementation of these systems, discuss operational challenges encountered during community-based eDNA surveys, and invite discussion on the future development of information infrastructures for knowledge sharing in participatory environmental DNA research.

Kaggle: Platform for addressing eDNA metabarcoding bioinformatics barriers in the global south

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Environmental DNA (eDNA) metabarcoding has emerged as a transformative approach for biodiversity monitoring and natural resource management, providing fast, accurate and cost-effective data. It is highly valuable in regions where traditional biodiversity surveys are logistically difficult, such as the global south, which hosts some of the world's most species-rich yet understudied ecosystems. Despite the growing potential of eDNA metabarcoding, its widespread application continues to be constrained by several infrastructural barriers. Limited access to resources for processing and analysis of high-throughput sequencing data are some of the major bottlenecks for the advancement and adoption of eDNA technologies into national biodiversity strategies and conservation policies.

To address ongoing barriers in eDNA metabarcoding research in the global south, we propose using Kaggle as a practical and scalable platform for data analysis. Its web-based environment eliminates the need for local software installation or specialized hardware, while providing a controlled yet flexible system for executing complex bioinformatic workflows. It handles key steps in the eDNA bioinformatics pipeline from data storage and file transfer to computationally demanding tasks such as denoising, clustering, and taxonomic assignment. Its notebook-based coding environment further supports clear workflow documentation, version tracking, and easy sharing of results.

Using this platform, we have demonstrated that with appropriate instructional design and cloud-based tools, researchers can effectively conduct eDNA-based biodiversity assessments even in low-resource settings. In doing so, it lays the groundwork for collaborative eDNA research and the co-production of regional or global biodiversity knowledge and supports broader capacity building in the global eDNA research community.

【P-04】

市民科学による環境 DNA 調査は沿岸魚類群集構造をどの程度正確に捉えられるか — 研究者調査との比較 —

Reliability of Citizen Science Environmental DNA Surveys for Assessing Coastal Fish Community Structure

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近年、市民科学を活用した eDNA 調査は広域モニタリング手法として期待されている。一方、市民科学による eDNA データの信頼性は十分に検証されていない。本研究では、2020～2023 年にアースウォッチ・ジャパンが日本沿岸で実施した eDNA 調査データを用い、市民科学参加者と研究者が採取した魚類群集構造を比較した。Bray-Curtis 距離に基づく部分距離ベース冗長分析 (partial dbRDA) を用い、海面水温、夜間光強度、および distance-based Moran's eigenvector maps (dbMEM) による環境・空間要因を条件変数として、採水者区分の影響を評価した。その結果、採水者区分は魚類群集構造に有意な影響を及ぼさなかった。以上より、採水者区分は魚類群集構造に影響を及ぼさず、市民科学参加者と研究者は同等の魚類群集データを取得できる可能性が示された。

Citizen science-based environmental DNA (eDNA) surveys have emerged as a promising approach for large-scale biodiversity monitoring. However, the reliability of eDNA data collected by citizen scientists remains insufficiently evaluated. In this study, we analyzed coastal eDNA survey data collected by Earthwatch Japan between 2020 and 2023 to compare fish community composition between samples collected by citizen scientists and professional researchers. Fish community composition was analyzed using partial distance-based redundancy analysis (partial dbRDA) based on Bray-Curtis dissimilarity, with sea surface temperature (SST), nighttime light intensity (NTL), and spatial variables represented by distance-based Moran's eigenvector maps (dbMEM) included as conditioning variables. Participant type did not have a significant effect on fish community composition. These results indicate that participant type had little influence on fish community composition and suggest that citizen scientists can obtain fish community data comparable to those collected by professional researchers.

【P-05】

環境 DNA の調査研究の地域ベースの教育、観測、実践、人材育成の大学研究室における現状と展望

The current state and outlook of regional-based education, observation, practice, and capacity building on environmental DNA in university laboratories

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環境DNAは、生物学、生態系の調査研究の強力な方法論で発展途上にある。現場での試料採取からバイオ技術、データ解析まで広い範囲をカバーする必要があるが、大学研究室は、大学の従来の基礎的な研究教育だけでなく、学校教育や地域社会との連携、企業との共同研究も行っている。データ利用の推進が言われる一報で、調査の現場の管理、事務作業などのロジスティック領域の重要性が看過されがちである。地域の視点から教育、安定的な観測、実践、人材育成の現状と展望を、九州地域を中心にした事例から論ずる。これは大学に限らず、調査会社などでも同様な状況にあり、問題提起と改善に向けた動きを進めたい。

Environmental DNA is an emerging, powerful methodology for studying biology and ecosystems. It requires covering a wide range, from field sampling to biotechnology and data analysis. But university laboratories not only handle traditional basic research and education, they also collaborate with schools, local communities, and companies. While people often talk about promoting data use, the importance of logistics like field management and administrative work tends to be overlooked. From a regional perspective, this paper discusses the current situation and future outlook of education, stable monitoring, practice, and human resource development, focusing on examples from the Kyushu region. This situation isn't limited to universities—survey companies face similar issues—and we want to push forward ideas and actions for addressing and improving them.

【P-06】

国立・地方環境研究所の連携による環境 DNA モニタリング手法の開発と普及

Development and dissemination of eDNA monitoring methods through collaboration among national and regional environmental research institutes

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国・地域レベルの環境行政に環境 DNA 分析を導入することは、生物多様性のモニタリングや保全の一層の推進につながる。本発表では、「地方公共団体環境研究機関等と国立環境研究所との共同研究」の枠組みの下で行われた、環境 DNA 分析手法の開発と普及に関する取り組みを紹介する。淡水環境において水生昆虫と脊椎動物を効率的に検出するメタバーコーディング手法の開発を進め、プライマー性能の評価や種検出の季節性の検証、DNA 配列情報が不足している水生昆虫類を中心としたリファレンスデータベースの整備を行った。さらに、技術交流会やハンズオン講習会を実施し、参加機関の間での情報交換や技術向上の機会を設けた。こうした中で、地方環境研究所において環境 DNA 分析の実践を妨げる様々な要因も浮き彫りとなった。本発表では、地域環境行政への環境 DNA 分析の効果的な導入に向けた課題と解決策について考察する。

Integrating eDNA analysis into environmental administration at the national and regional levels has the potential to enhance biodiversity monitoring and conservation. This presentation introduces initiatives to develop and disseminate eDNA analysis methods conducted as part of the Joint Research Program between Local Government Environmental Research Institutes and the National Institute for Environmental Studies. We aimed to develop a metabarcoding method for efficient detection of benthic invertebrates and vertebrates in freshwater ecosystems. The study evaluated primer performance, assessed seasonal variation in species detection, and expanded a reference database focusing on aquatic insects, for which DNA reference sequences remain insufficient. Technical exchange meetings and hands-on workshops were also organized to promote information sharing and capacity building among participating institutions. These activities revealed several barriers to implementing eDNA analysis at local environmental research institutes. We discuss these challenges and potential solutions for effectively integrating eDNA analysis into regional environmental administration.

Advancing aquatic biomonitoring in Ireland through DNA-based methods

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HydroGen is an EPA-funded project investigating the integration of environmental DNA (eDNA) metabarcoding into routine biodiversity and water quality monitoring in Ireland.

Results from a two-year monitoring programme are presented for aquatic macroinvertebrates, other metazoan taxa, and microbial communities. DNA metabarcoding recovered a diverse range of taxa relevant to ecological assessment and enabled direct comparisons with traditional morphological surveys. Microbial community analyses identified patterns associated with environmental conditions and potential contamination sources, highlighting the potential of molecular approaches for water quality assessment and microbial source tracking. The study also evaluates the influence of methodological choices, including filtration strategies and laboratory workflows, on biodiversity recovery.

Overall, the project demonstrates the value of DNA-based methods in complementing conventional monitoring and provides practical insights to support their integration into routine aquatic monitoring programmes in Ireland.

【P-08】

河川水辺の国勢調査への環境 DNA 実装 令和元年から 7 年間の取り組み ～河川版～

Implementing Environmental DNA into the National Census on River Environments

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国土交通省が行う河川水辺の国勢調査に、令和 8 年度より環境 DNA 調査が実装され、河川では流域を俯瞰する生物情報を得るための多地点調査が、ダム湖では湖内の生物相の変化をいち早く捉えるために高頻度な調査が行われる。いずれも、環境 DNA の「現地調査の負担が小さい」という特性を活かした導入方法となっている。本件では、導入方法の検討を行う中で、土木研究所が国土交通省および地方整備局と連携して進めてきた河川の全国調査について報告する。

Environmental DNA surveys have been implemented during the National Census of River Environments conducted by the Japan's Ministry of Land, Infrastructure, Transport and Tourism since fiscal year 2026. Rivers were surveyed at multiple points to obtain a bird's-eye view of the watershed biological information, while dam lakes were frequently studied to quickly capture biotic changes within the lake. Both methods utilize the "minimal effort for on-site surveys" characteristic of environmental DNA. This report reviews the nationwide surveys on river conducted by the Civil Engineering Research Institute in collaboration with the Ministry of Land, Infrastructure, Transport and Tourism and the Regional Development Bureaus to assess implementation methods.

【P-09】

商船を利用した環境 DNA 観測の可能性

Potential of eDNA Monitoring by Utilizing Merchant Vessels

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近年、海洋生態系の保全や水産資源の持続的利用への関心の高まりを背景に、広域かつ継続的な海洋生物観測の需要が高まっている。環境 DNA 観測は、従来の採捕調査等よりも簡便かつ網羅的に生物分布を把握できる手法であり、沿岸域を中心に多くの調査が行われている。しかし、外洋域は地表の大部分を占めるにも関わらず、調査コストの高さから生物分布に関するデータは依然として不足している。

本研究では、日常的に外洋域を航行する商船を利用して環境 DNA 観測を行い、広域的な生物分布の把握を試みる。また、環境 DNA 観測における商船利用の実用可能性を検討する。2026 年 2-6 月に外航船舶を用いて公海上の 40 地点で採水した。採水地点は北太平洋、インド洋、南大西洋および北大西洋に位置し、サンプリング実績がない海域も含まれる。

今後さらに商船を活用したデータ収集を継続することで、全球規模の海洋生物分布の解明が期待される。

In recent years, the demand for broad-scale and continuous marine biodiversity monitoring has increased with growing interest in marine ecosystem conservation and the sustainable use of fishery resources. Environmental DNA (eDNA) monitoring has emerged as a simple and comprehensive approach for assessing species distributions compared with conventional capture-based surveys, and has been widely applied, particularly in coastal waters. However, although the open ocean covers most of the Earth's surface, data on marine species distributions remain limited because of the high costs associated with surveys.

In this study, we conducted eDNA monitoring utilizing merchant vessels that routinely navigate the open ocean to investigate broad-scale patterns of marine species distributions. We also evaluated the potential of utilizing merchant vessels as a platform for eDNA monitoring. Between February and June 2026, water samples were collected from 40 sites in international waters using ocean-going merchant vessels. The sampling sites were located in the North Pacific, Indian, South Atlantic, and North Atlantic Oceans, including areas where no previous eDNA sampling has been conducted.

Continued data collection using merchant vessels is expected to contribute to elucidating global patterns of marine species distributions.

【P-10】

環境 DNA を用いた流域網羅的な環境健全度評価に向けた基礎的検討

Basin Study for basin-wide biotic integrity evaluation using environmental DNA

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本研究では、環境 DNA 分析を用いて流域網羅的な環境健全度モニタリングを行い、環境健全度と環境要因との関係を検討することを目的とした。一級河川高津川を対象とし、流域網羅的に環境 DNA モニタリングを行い、環境 DNA 定量メタバーコーディングにより各魚種の環境 DNA 濃度を算出した。続いて、各魚種の環境 DNA 濃度を環境健全度指標である生物学的指数に変換し統合することで環境健全度スコアを算出し、統計解析を用いて環境健全度スコアと環境要因との関係を検討した。結果として、環境健全度が相対的に高い河川区間の環境条件が明らかとなった。

In this study, we conducted basin-wide environmental DNA monitoring and examined relationships between the biotic integrity and environmental factors in a river basin. We conducted eDNA sampling in the Takatsu River, the first-class river in Japan and calculated fish eDNA concentrations using quantitative eDNA metabarcoding. We converted the fish assemblage data into biotic integrity scores and examined relationships between the biotic integrity scores and environmental factors. As a result, we could identify the environmental conditions for the river segments with higher biotic integrity scores in the river basin.

【P-11】

海洋生態系の健全性評価に向けた東北沿岸 3 湾の長鎖メタゲノム解析

Long-read metagenomic analysis of three coastal bays in northeastern Japan toward assessment of marine ecosystem health

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海洋生態系の健全性評価には、物理化学的指標や生物指標など、さまざまなアプローチが検討されている。細菌から真核プランクトンまでの生物群集とその機能を包括的に捉えるメタゲノムデータも、健全性評価に資する情報源となる可能性がある。本研究では、その可能性を検討する基礎として、PlanDyO プロジェクトで 2025 年に女川湾、石巻湾、陸奥湾から得た Oxford Nanopore 長鎖メタゲノムデータを解析した。試料間で類似した出現パターンを示すコンティグ群、Kraken および MetaEuk で推定した属、KEGG Orthology に基づく遺伝子機能など 6 種類の解析単位について、各試料で検出された要素数を算出した。各指標は月別・観測地点別に概ね共通した変動を示し、一部は溶存酸素濃度と負の相関を示したが、その傾向は湾によって異なった。現在、真核生物と原核生物の寄与を分けて解析している。

Various approaches based on physicochemical and biological indicators have been explored for assessing marine ecosystem health. Metagenomic data that comprehensively capture biological communities and their functions, from bacteria to eukaryotic plankton, may provide an additional source of information for such assessment. As a basis for examining this possibility, we analyzed Oxford Nanopore long-read metagenomic data collected in 2025 from Onagawa Bay, Ishinomaki Bay, and Mutsu Bay through the PlanDyO project. We examined six types of sequence-derived features, including groups of contigs with similar occurrence patterns across samples, genera inferred using Kraken and MetaEuk, and gene functions defined by KEGG Orthology. For each feature type, we counted the number of elements detected in each sample. These measures showed broadly consistent variation across months and sampling stations. Some measures were negatively correlated with dissolved oxygen concentrations, although the patterns differed among bays. We are currently examining the respective contributions of eukaryotic and prokaryotic organisms.

【P-12】

環境 DNA を用いた氾濫原プールの環境健全度評価に向けた基礎的検討

Basic study for biotic integrity evaluation of floodplain pools using environmental DNA

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本研究では、環境 DNA 分析を用いて氾濫原プールの環境健全度を評価可能か検討することを目的とした。一級河川江の川の土師ダム下流に点在する氾濫原プールを対象とし、2025 年の 11 月に環境 DNA 分析のための採水と電気ショッカーを用いた魚類の採捕調査を行った。採水試料は、室内でろ過・抽出・環境 DNA 定量メタバーコーディングを行い各魚種の環境 DNA 濃度を算出した。続いて、各魚種の環境 DNA 濃度および採捕データを環境健全度指標である生物学的指数に変換し、統計解析を用いて環境 DNA 分析と採捕調査による生物学的指数の関係を検討した。結果として、環境 DNA 分析と採捕調査による生物学的指数との間に顕著な正の相関関係がみられ、環境 DNA を用いて氾濫原プールの環境健全度評価が可能になることが示された。

In this study, we examined if the evaluation of biotic integrity in floodplain pools is possible using environmental DNA (eDNA). We collected water samples for eDNA analyses and fishes by electrofishing in floodplain pools in the downstream of the Haji Dam in Gonokawa River, the first-class river in Japan, in November 2025. We calculated the eDNA concentrations of each fish via filtration, extraction, and quantitative eDNA metabarcoding. Then, we converted the fish assemblage data of the eDNA analysis and electrofishing into the biotic integrity scores and compared the scores from the two sampling methods. As a result, the eDNA-based biotic integrity scores were significantly and positively correlated with the biotic integrity scores of the electrofishing, indicating that it is possible to evaluate the biotic integrity in the floodplain pools using eDNA.

【P-13】

水環境における高病原性鳥インフルエンザウイルスモニタリング手法の開発

Development of a monitoring system for highly pathogenic avian influenza virus in water environments

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高病原性鳥インフルエンザ（HPAI）は養鶏産業に甚大な被害を及ぼすため、渡り鳥を介したウイルス侵入の早期発見が重要である。本研究では、環境水中の HPAI ウイルスを検出する新規ワークフロー（濃縮法 QuickConc と核酸抽出法 COPMAN の組み合わせ）を検証した。スパイク試験の結果、本手法は従来法と比較して qPCR による A 型インフルエンザウイルス（AIV）の検出において 100 倍以上の感度を示した。2024 年 6 月～2025 年 5 月に宮崎市で実施した調査では、渡り鳥の飛来増加に伴い AIV の M 遺伝子の検出頻度および量が増加した。近隣養鶏場でも HPAI 発生前に H5 遺伝子を検出することに成功した。他地点での調査でも M および H5 遺伝子の検出が可能であり、HA 開裂部位の解析により高病原性型であることを確認した。本ワークフローは HPAI モニタリングに有用であり、早期警戒システムとして活用が期待される。

The highly pathogenic avian influenza virus (HPAIV), H5 or H7 subtype, threatens wildlife and livestock. Migratory waterfowl are its main reservoirs, so a more effective monitoring method is necessary to reduce HPAIV damage. Here, we report a novel workflow for detecting HPAIV in environmental water with high sensitivity and its field applicability. This workflow integrates three components: nucleic acid concentration from a liter of water with QuickConc, extraction using COPMAN, and gene detection via RT-preamplification-qPCR. Spike experiments showed our workflow was 100 times more sensitive than a conventional method. Using this method, we conducted a one-year longitudinal monitoring of AIV and HPAIV dynamics in a lake in Miyazaki Prefecture, where migratory birds stay during autumn and winter. No viral genes were detected in summer, although the M gene, which is pan Influenza A virus marker, was firstly identified in early September. Then, its detection frequency and amount increased in parallel with the growing number of migratory birds. Furthermore, the H5 gene, HPAIV marker, was detected prior to HPAIV cases in surrounding areas. We also tested our workflow in seven lakes in other area of Japan during winter. The M gene was repeatedly detected in all lakes, and the H5 gene was spontaneously detected in three of them. Moreover, we identified some detected H5 genes as the highly pathogenic type through direct sequencing of the HA cleavage site. This workflow enables efficient HPAIV monitoring in water environments, useful for raising awareness about potential cases.

【P-14】

ヴィクトリア湖畔におけるマンソン住血吸虫の季節分布および分布規定要因

Seasonal distribution and environmental drivers of *Schistosoma mansoni* inferred by eDNA analysis in Lake Victoria

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住血吸虫症は、淡水性巻貝から水中に放出される住血吸虫のセルカリアが経皮感染して発症する寄生虫感染症であり、多くの国で深刻な公衆衛生問題となっている。これまでの研究では、降雨に伴う環境変化により住血吸虫や中間宿主巻貝の分布は変動すると考えられるが、広域かつ複数季節で評価した大規模研究は少ない。本研究では、ヴィクトリア湖の Rusinga 島（ケニア）を含む湖畔 47 地点において、2025 年 6～7 月、2025 年 11～12 月、および 2026 年 2 月の計 3 回にわたり環境 DNA 調査を実施し、計 423 検体を採取した。さらに、eDNA 濃度、環境要因（水温、pH など）、およびサンプリング地点から半径 50 メートル圏内にある住宅数との関連性を分析した。これにより、住血吸虫感染症のリスクが高い地域や時期を特定し、その季節的分布を左右する要因を解明することが期待される。

Schistosomiasis is a parasitic disease caused by percutaneous infection with *Schistosoma* cercariae released by certain species of freshwater snails, posing a significant public health challenge in many countries. Although previous studies suggest that the distribution of schistosomes and their intermediate host snails fluctuates in response to environmental changes associated with rainfall, large-scale studies evaluating these dynamics across broad geographical areas and multiple seasons remain limited. In this study, environmental DNA (eDNA) surveys were conducted at 47 lakeshore sites, including Rusinga Island in Lake Victoria, Kenya, during June–July 2025, November–December 2025, and February 2026, yielding a total of 423 water samples. The seasonal spatial distribution of *Schistosoma mansoni* eDNA concentrations was evaluated using quantitative real-time PCR. Furthermore, the relationship between *S. mansoni* eDNA concentrations, environmental factors (e.g., water temperature, pH), and the number of houses within a 50-m radius of each sampling site were analyzed. The findings of this study are expected to contribute to the identification of high-risk areas and periods for *Schistosoma* infection and to improve understanding of the determinants of its seasonal distribution.

Development of highly sensitive multi-marker qPCR assay for the eDNA detection of *Schistosoma haematobium*

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Schistosoma haematobium is a trematode parasite that infects blood vessels and cause schistosomiasis. Humans become infected when they come into contact with freshwater containing cercariae released by infected intermediate-hosts snails. Rapid identification of contaminated water bodies is therefore critical for controlling disease transmission. Traditional snail monitoring is costly and labor-intensive, rendering them unsuitable for large-scale, real-time monitoring. In contrast, eDNA analysis allows for low-cost, real-time monitoring with minimal labor for detecting *S. haematobium*. To date, most eDNA assays for *Schistosoma* species have shown limited sensitivity and low detection rates in environmental samples. The objective of our study was to develop a highly sensitive qPCR assay targeting *S. haematobium* for eDNA detection. To improve detection sensitivity, we developed a multi-marker assay incorporating the 16S, COI, and ITS2 regions, and compared its detection sensitivity with that of single-marker assays. For field evaluation, water samples were collected from 40 sites across the Kwale County of Kenya. We are currently preparing to test the assay we developed using field samples. The results will subsequently be analyzed using Generalized Linear Mixed Models (GLMM).

Comparison of eDNA and PMA-based iDNA Detection across Filter Pore Sizes for Harmful Algal Bloom Species Using qPCR

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1. Gyeongsang National Univ.

Environmental DNA (eDNA) and intracellular DNA (iDNA) are increasingly used for monitoring harmful algal bloom (HAB) species; however, the influence of filter pore size on DNA recovery remains poorly understood. This study evaluated the effects of four filter pore sizes (0.2, 1.2, 10, and 30 μm) on the detection of three HAB taxa (Alexandrium, Pseudo-nitzschia, and Margalefidinium) using quantitative PCR (qPCR). Seawater samples collected from a coastal site were filtered through each pore size, and each filter was divided into two fractions for total eDNA and PMA-treated iDNA analyses. DNA copy numbers were quantified using species-specific qPCR assays and compared with microscopic cell counts.

Across all target taxa, DNA copy numbers were consistently higher in the eDNA fraction than in the PMA-treated iDNA fraction, indicating effective exclusion of extracellular or membrane-compromised DNA following PMA treatment. Alexandrium exhibited the highest DNA copy numbers in the 10 μm fraction for both eDNA and iDNA, whereas Pseudo-nitzschia showed relatively high eDNA abundance across the 0.2–10 μm fractions and the highest iDNA abundance in the 1.2 μm fraction. Margalefidinium was detected by qPCR despite the absence of microscopic observations. The relationship between microscopy and qPCR varied among species and filter fractions. The highest coefficients of determination (R^2) were observed in the 30 μm iDNA fraction for Alexandrium ($R^2 = 0.95$) and in the 10 μm eDNA ($R^2 = 0.94$) and 10 μm iDNA ($R^2 = 0.95$) fractions for Pseudo-nitzschia. These findings demonstrate that DNA recovery and agreement with microscopic observations are species-dependent and influenced by filter pore size and DNA fraction. The results provide practical guidance for optimizing qPCR-based HAB monitoring using eDNA and PMA-based iDNA.

Evaluation of Cell- and DNA-Based Standard Curves for PMA-qPCR Quantification of Viable Marine Phytoplankton

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Conventional quantitative PCR (qPCR) enables sensitive and specific quantification of phytoplankton but cannot distinguish DNA from viable and non-viable cells. Accurate quantification of viable cells is particularly important for marine phytoplankton associated with harmful algal blooms and aquaculture impacts. This study evaluated the applicability of propidium monoazide (PMA)-qPCR for the selective quantification of viable phytoplankton using *Chaetoceros muellerii*, *Prymnesium parvum*, and *Heterosigma akashiwo*.

Cell-based (cell dilution) and DNA-based (DNA dilution) standard curves were compared using qPCR and PMA-qPCR under three viability conditions: 100% viable cells, a 1:1 mixture of viable and Triton X-100-treated membrane-compromised cells (50%), and 100% membrane-compromised cells (0%).

Under the 100% viable cell condition, both qPCR and PMA-qPCR generated standard curves using the cell dilution method; however, the cell-based approach showed lower linearity and reproducibility than the DNA dilution method. In contrast, DNA dilution produced stable C_q values and robust standard curves across the dilution series, demonstrating greater suitability for standard curve construction. Under the 50% and 0% viability conditions, qPCR detected DNA from both viable and membrane-compromised cells, whereas PMA-qPCR effectively suppressed amplification of DNA from Triton X-100-treated cells, resulting in no detectable standard curve.

These findings demonstrate that PMA-qPCR enables selective quantification of viable marine phytoplankton and that DNA dilution provides superior linearity and reproducibility for standard curve construction. This approach provides a technical basis for viability-based quantification and monitoring of harmful marine phytoplankton.

【P-18】

外来珪藻 *Cymbella janischii* の繁茂要因を環境 DNA と流域空間解析から探る

Exploring the Factors Driving blooms of an Invasive Diatom *Cymbella janischii* Using Environmental DNA and Watershed Spatial Analysis

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河川水の環境 DNA 分析と流域の空間解析は、広域の生物分布と環境を効率的に把握することが可能なベストコンビである。本研究では、外来珪藻 *Cymbella janischii* の繁茂に関与する流域の環境因子を解明するため、繁茂・非繁茂河川について、環境 DNA 採水場所を集水地点とする流域内（集水域）の空間解析を試みた。解析は地理情報システム（GIS）を用い、建物、河川構造物、コンクリート被覆、森林・農地の種類、地質、日照量等の環境因子について検討した。発表では、解析の進捗と得られた知見、今後の課題について議論する。

Environmental DNA (eDNA) analysis of river water and watershed spatial analysis are a powerful combination for efficiently assessing biological distributions and environmental conditions over broad areas. To identify watershed environmental factors associated with the blooms of the invasive diatom *Cymbella janischii*, we conducted spatial analyses of catchment area to eDNA sampling sites in rivers where the species either forms extensive blooms or occurs without substantial blooming. The analyses were performed using a geographic information system (GIS), and environmental factors currently under investigation include buildings, river structures, concrete-covered areas, forest and farmland types, geology, and solar radiation. The presentation will discuss the progress of the analyses, findings obtained to date, and future challenges.

Comparison of Coastal Phytoplankton Communities Between Environmental DNA and Propidium Monoazide-Based Intracellular DNA Metabarcoding in Korean Coastal Waters

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Environmental DNA (eDNA) metabarcoding is widely used to assess phytoplankton communities, but eDNA includes both intracellular DNA from living cells and extracellular or dead-cell DNA, which may not accurately reflect viable assemblages. Propidium monoazide (PMA) selectively binds extracellular and membrane-compromised-cell DNA, blocking its amplification and enabling preferential detection of intracellular DNA (iDNA). Direct comparisons between eDNA- and PMA-based iDNA metabarcoding for phytoplankton remain limited. This study compared eDNA and iDNA-based phytoplankton communities at three Korean coastal sites: Unjin and Seogwipo (Jeju Island, April 2026) and Tongyeong (May 2026). Surface seawater (3 L) was filtered through 1.2 μm nylon membranes; each filter was split into two portions for eDNA extraction and PMAxx (25 μM) treatment prior to iDNA extraction. The 18S rRNA V4 region was sequenced on Illumina, with ASVs generated via DADA2 and taxonomy assigned using SILVA 138. Distinct shifts were observed between eDNA and iDNA at all sites. At Unjin, *Gyrodinium* dominated eDNA (~60%) but declined sharply in iDNA, while *Tetraselmis* became dominant (~27%). At Tongyeong, *Akashiwo* dominated eDNA (~73%) but dropped to ~27% in iDNA, with *Biecheleria* and *Karlodinium* increasing in relative abundance. At Seogwipo, diatoms (*Minidiscus*, *Chaetoceros*) remained dominant in both fractions, though their relative proportions and *Prorocentrum* abundance shifted following PMA treatment. These results indicate that PMA-based iDNA metabarcoding can substantially alter perceived community composition compared to conventional eDNA, particularly by reducing the apparent dominance of taxa likely represented by extracellular or non-viable DNA, and may better reflect viable phytoplankton assemblages. Further validation using taxon-specific qPCR and biological replicates will be required to confirm these preliminary observations.

Temperature-Sensitive Predatory Zooplankton *Leptodora* sp. in Korean Lakes: Morphological and Molecular (COI) Identification for Resolving Recent Taxonomic Uncertainty

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The genus *Leptodora* is a predatory cladoceran that feeds on rotifers, small cladocerans, and medium-large cladocerans. It occurs predominantly in summer, reflecting a biological characteristic in which both prey density and its own growth rate through molting increase depending on water temperature. Accordingly, *Leptodora* carries ecological significance as an intermediate predator within freshwater food webs and as an indicator species of water temperature change. The genus had long been regarded as monotypic, represented solely by *L. kindtii*, until *L. richardi* was described in 2009 based on morphological differences between Asian and European populations. Subsequent molecular phylogenetic analysis indicated that these two represent distinct lineages. Korea lies along a biogeographically important pathway connecting the Eurasian continent to the isolated island of Honshu, Japan; nevertheless, no genetic studies have been conducted on Korean *Leptodora* populations. This study therefore examined the distribution, morphological identification, and genetic analysis of *Leptodora* populations in Korea. Occurrence records were compiled from major Korean lakes, and specimens from representative lakes were identified as *L. richardi* or *L. kindtii* based on the presence or absence of setules on the anterior distal seta of the first thoracic limb (tl I). Results were then compared with mitochondrial (COI) analysis to evaluate the limitations of current morphological identification, and to determine the phylogenetic position of Korean populations and their genetic similarity to Chinese and Japanese populations. This study is expected to help fill the distribution gap for *Leptodora* within East Asia and provide data for future research on zooplankton dispersal and phylogeography.

PHYTOPLANKTON COMMUNITY COMPOSITION AND SPATIAL HETEROGENEITY ACROSS WET AND DRY SEASONS IN A TROPICAL VOLCANIC LAKE REVEALED BY 23S eDNA METABARCODING

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Lake Batur, a deep tropical volcanic lake in Bali, has traditionally been classified as a monomictic system, yet how this mixing regime shapes seasonal phytoplankton community dynamics remains poorly resolved. Here, high-throughput 23S rRNA eDNA metabarcoding was used to characterize phytoplankton community composition and evaluate spatial and vertical heterogeneity between the rainy (February) and dry (August) seasons. Water samples were collected in triplicate from 12 monitoring stations at two depths (5 and 25 m), yielding 144 filter samples across both seasons. Environmental DNA was amplified using the P23MISQF1/P23MISQR1 primer pair and sequenced on the Illumina MiSeq platform, followed by processing into amplicon sequence variants (ASVs). Community dissimilarity was assessed using Bray–Curtis distances, Principal Coordinate Analysis (PCoA), and PERMANOVA. Phytoplankton communities showed distinct seasonal patterns: during the rainy season, low vertical Bray–Curtis dissimilarity (0.23), overlapping PCoA ordinations, and non-significant vertical differentiation (PERMANOVA, $p = 0.074$, $R^2 = 0.086$) indicated vertically homogeneous communities across the lake. In contrast, the dry season showed pronounced spatial heterogeneity, with vertical dissimilarity ranging from 0.026–0.432 and significant differentiation between depths (PERMANOVA, $p = 0.001$, $R^2 = 0.326$). Notably, stations with the lowest vertical dissimilarity coincided with documented mass fish kill locations, suggesting that phytoplankton eDNA signatures can reveal localized hydrodynamic disturbances. These findings demonstrate that 23S eDNA metabarcoding provides a sensitive approach for resolving seasonal phytoplankton community dynamics, offering new insight into mixing processes and ecological risk assessment in tropical volcanic lake ecosystems.

【P-22】

東北沿岸 3 湾から検出された *Alexandrium* 属完全長 18S rDNA 配列の系統解析

Phylogenetic analysis of full-length 18S rDNA sequences of *Alexandrium* recovered from three bays along the northeastern coast of Japan

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渦鞭毛藻 *Alexandrium* 属には麻痺性貝毒の原因となる種が含まれるため、沿岸域における同属の系統群構成は、貝毒原因種のモニタリングを検討する上で重要な基礎情報となる。沿岸プランクトン群集の長鎖メタエピゲノミクス研究基盤 PlanDyO では、東北沿岸 3 湾で継続的に海水を採取し、長鎖メタゲノム解析を行っている。本研究では、2024 年 4 月から 2026 年 5 月に女川湾、石巻湾、陸奥湾で採取した海水サンプルを対象に、*Alexandrium* 属の系統群構成を解析した。PR2 に登録された *Alexandrium* 属 13 種 83 配列を参照として完全長 18S rDNA 配列 292 本を取得し、外群を含む系統解析を行った。その結果、取得配列は、*A. catenella* に対応する 2 群と、*A. pseudogonyaulax*、*A. insuetum*、*A. pacificum* に対応する各 1 群の計 5 系統群を形成した。

The dinoflagellate genus *Alexandrium* includes species that cause paralytic shellfish poisoning. Characterizing the phylogenetic composition of this genus in coastal waters can therefore provide fundamental information for monitoring PSP-causing species. PlanDyO, a platform for long-read metaepigenomics of plankton communities in coastal marine ecosystems, continuously collects seawater samples and conducts long-read metagenomic analyses in three bays along the northeastern coast of Japan. In this study, we analyzed the phylogenetic composition of *Alexandrium* using seawater samples

【P-23】

沿岸海洋生態系におけるプランクトン群集の長鎖メタエピゲノミクス研究 基盤 PlanDyO

PlanDyO platform for long-read metaepigenomics of plankton communities in coastal marine ecosystems

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沿岸海洋生態系が環境変化にどのように応答するかを理解するには、生物群集の構成変化だけでなく、群集を構成する生物の機能や生理状態が環境条件に応じてどのように変化するかを捉える必要がある。我々は、沿岸海洋生態系のプランクトン群集を対象に、長鎖メタエピゲノミクスによる定期観測を 2024 年に開始した。これまでに女川湾、石巻湾、陸奥湾の約 100 サンプルを解析し、その結果を PlanDyO データベースとして WPI-AIMEC 内に公開している。データベースでは、長鎖リードのアセンブリ、分類群組成、推定遺伝子機能、DNA メチル化パターン、CTD 観測値、細胞数を絶対定量したフローサイトメトリーデータ、定性的参照用の顕微鏡画像を閲覧できる。PlanDyO は、プランクトン群集の環境変化への応答を解析する基盤を提供する。データベースは 2027 年 3 月までの一般公開を予定している。

Understanding how coastal marine ecosystems respond to environmental change requires examining not only shifts in community composition but also how the functions and physiological states of constituent organisms vary with environmental conditions. In 2024, we launched PlanDyO, a project conducting regular long-read meta-epi-genomic monitoring of plankton communities in coastal marine ecosystems. To date, we have analyzed approximately 100 samples from Onagawa, Ishinomaki, and Mutsu Bays. The resulting data are available internally to WPI-AIMEC researchers through the PlanDyO database. The database provides long-read assemblies, taxonomic profiles, predicted gene functions, DNA methylation patterns, CTD observations, flow cytometry data for absolute quantification of cell abundance, and microscopy images for qualitative reference. PlanDyO provides a platform for examining how plankton communities respond to environmental change. Public release of the database is planned by March 2027.

Detection of overlooked microbial interactions under field conditions using multi-taxa, quantitative environmental DNA analysis

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Understanding microbial interactions in open field systems is challenging due to complex interactions among biotic and abiotic variables. Many population-level interspecific interactions have been overlooked by conventional methods like microscopic observation. This study proposes a framework to detect these hidden interactions in freshwater ecosystems by combining nonlinear time-series analysis, high-frequency quantitative environmental DNA (eDNA) monitoring, and field manipulation experiments. Our approach involved four steps: (1) inferring interspecific interactions from daily eDNA time series using causal analysis; (2) isolating and culturing a candidate causal taxon (a chytrid species); (3) performing field manipulations by introducing the cultured species; and (4) evaluating community shifts. First, the time series analysis of daily quantitative eDNA data from artificial rice fields in 2017 in Shiga, Japan identified potential interactions between chytrids and other microbes. In 2022, after isolating local chytrid strains, we inoculated them into the experimental plots, which resulted in a sharp increase in target chytrid eDNA concentrations. After the manipulation, specific algal abundance was maintained at low levels, while a Pseudomonadota bacterial species increased with a time delay, indicating a facilitative interaction. Re-analysis of the extensive time series taken in 2017 confirmed that the temporal dynamics observed in 2017 supported the results of the manipulative experiments. These results demonstrate that integrating time-series analysis and field manipulation can successfully detect overlooked microbial interactions in nature. This data-driven framework provides a powerful tool to uncover hidden microbial interactions under complex, real-world conditions.

【P-25】

環境 DNA を用いたダム上下流における魚類多様性の把握に関する基礎的検討

Basic study on fish diversity assessment upstream and downstream of dam using environmental DNA

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一級河川江の川の浜原ダム上下流を調査地とし、本川下流、本川上流、支川下流、支川上流の4グループを設定した。各グループで10地点ずつ調査地点を設定し、2026年5月に環境DNA分析のための採水を行い、定量メタバーコーディング法によって各魚種の環境DNA濃度を算出した。さらに、検出種数と各魚種の環境DNA濃度に基づき、地点ごとの検出種数 (α 多様性)、地点間の群集構造の違い (β 多様性)、各グループ全体の検出種数 (γ 多様性) を算出し、グループ間で比較検討した。その結果、本川では地点当たり20～30種が検出された一方、ダム上流の支川では5～25種と有意に少なかった。また、ダム上流の支川では β 多様性が最も高く、地点間で魚類群集構造が大きく異なった。さらに、ダム下流では回遊魚や周縁魚を含む40種が確認され、 γ 多様性が最も高かった。

This study aimed to evaluate differences in fish diversity upstream and downstream of a dam using environmental DNA (eDNA) analysis. The study was conducted around Hamahara Dam in the Gonokawa River, Japan. Four groups were established: the downstream mainstream, upstream mainstream, downstream tributaries, and upstream tributaries. Ten sampling sites were selected for each group, and water samples were collected in May 2026 for eDNA analysis. Fish eDNA concentrations were quantified using the quantitative metabarcoding method. Based on the number of detected species and fish eDNA concentrations, site-level species richness (α -diversity), differences in fish assemblage structure among sites (β -diversity), and total species richness within each group (γ -diversity) were evaluated and compared among the four groups. The results showed that 20–30 species were detected per site in the mainstream, whereas only 5–25 species were detected in the upstream tributaries, indicating the tributaries had significantly lower α -diversity than the mainstream. The upstream tributaries exhibited the highest β -diversity, suggesting the greatest spatial variation in fish assemblage structure among sites. In addition, the downstream area showed the highest γ -diversity (a total of 40 species detected) including migratory and peripheral fish species.

【P-26】

環境 DNA 定量メタバーコーディングを用いた佐波川における堰が魚類へ与える影響の基礎的検討

Preliminary study on the impact of weirs on fishes in the Saba River using quantitative environmental DNA metabarcoding

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山口県の一級河川である佐波川の国管理区間（河口から -1.2 ～ 26.2 km）には 15 基の堰が設置されている。これらの堰は利水や治水に重要な役割を果たしている一方で、堰は上流に湛水域を形成し、上流から下流への土砂輸送の連続性を低下させるため、河道形状などの物理環境を改変し、生物の生息環境に影響を及ぼすことが報告されている。そこで本研究では、佐波川の国管理区間において堰の直上、直下を含む 28 地点で採水を行い、環境 DNA 定量メタバーコーディングを用いることで、堰が密に設置された国管理区間での魚類の分布を把握し、堰が魚類に与える影響について検討した。

Fifteen weirs have been installed along the nationally managed section (extending from -1.2 km to 26.2 km upstream from the river mouth) of the Saba River, the first-class river in Yamaguchi Prefecture, Japan. While these weirs play a crucial role in water utilization and flood control, they also create impoundments upstream and disrupt the continuity of sediment transport from upstream to downstream. Such alterations to physical environments including river channel morphology have been reported to impact aquatic habitats. In this study, we collected water samples from 28 locations within the nationally managed section of the Saba River including points immediately upstream and downstream of the weirs and employed quantitative environmental DNA metabarcoding to map fish distribution and examine the impact of the weirs on fish populations in this area where weirs are densely situated.

【P-27】

種の生態から紐解く河川魚類群集の季節性：群集階層モデルによるアプローチ

Unravelling the seasonality of riverine fish detection using hierarchical modelling of species communities

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環境 DNA 調査は魚類群集の季節変化を鋭敏に捉えるが、その変化は水温などの環境要因と関連付けられることが多い。しかし実際には、流れや各種の生活史も検出パターンに影響するため、それぞれの寄与を切り分けなければ季節性を適切に解釈できない。本研究では京都府伊佐津川水系の 12 地点で三年間の四季調査を行い、155 試料を MiFish 法により分析した。種の存在の規定要因を明らかにするため、生態形質と空間・環境条件を結ぶ群集階層モデルを適用した。種検出パターンを最も強く規定したのは河口からの位置であり、季節がこれに次いだ。さらに種ごとの季節的な検出パターンは、各種の産卵期を組み込んだモデルで最もよく説明され、回遊型と底生・遊泳性のみを形質として用いた場合の約 1.4 倍の説明力を示した。群集の季節変化を理解するには、空間と環境による二元的枠組みではなく、種ごとの生活史を組み込んだ階層的アプローチが求められる。

Environmental DNA (eDNA) surveys sensitively capture seasonal variation in fish communities. While this variation is commonly linked to environmental conditions such as water temperature, it is also shaped by longitudinal position within rivers and species-specific life histories. Disentangling the relative contributions of these factors is essential for understanding seasonal community dynamics.

We conducted seasonal surveys over three years at 12 sites in the Isazu River system, Kyoto Prefecture, Japan, and analysed 155 samples by MiFish metabarcoding. To identify the determinants of species occurrence, we applied Hierarchical Modelling of Species Communities (HMSC), which links species ecological traits with spatial and environmental covariates.

Distance from the river mouth was the strongest determinant of detection patterns, followed by season. Species-specific seasonality in detection was best explained by the model incorporating spawning season, whose explanatory power (coefficient of discrimination) was 1.4 times that of the model using migratory type and pelagic/demersal habit alone. Understanding seasonal changes in community composition therefore requires more than a framework based solely on space and environment: a hierarchical approach such as HMSC that incorporates the life history of each species is needed.

【P-28】

ロングリード環境 DNA メタバーコーディングによる魚類群集の種多様性と種内遺伝的多様性の同時評価

Simultaneous assessment of species and intraspecific genetic diversity in fish communities using long-read environmental DNA metabarcoding

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生物多様性評価は種レベルの多様性に依存してきたが、昆明・モンリオール生物多様性枠組に示されるように遺伝的多様性の重要性も認識されつつある。環境 DNA 解析で主流の短鎖アンプリコン（400bp）は情報量が限られ、近縁種の識別や種内多型の評価に制約がある。本研究では、種多様性と種内遺伝的多様性を同時に評価するロングリード環境 DNA メタバーコーディング手法を開発した。ミトコンドリア Cytb-12S rRNA 間の 2-3 kbp 領域を増幅する新規ユニバーサルプライマーを設計し、複数系統のメダカ・イトヨを用いた水槽実験、琵琶湖博物館の展示水槽、および琵琶湖の野外調査で検証した。水槽実験ではハプロタイプを組織 DNA と照合し、展示水槽・野外では短鎖（MiFish-U）解析と検出種数を比較した。本手法は種間・種内の情報への同時アクセスを可能にし、高解像度な生物多様性モニタリングの基盤となることが期待される。

Biodiversity assessment has long relied on species-level diversity, yet the importance of genetic diversity is increasingly recognised, as reflected in the Kunming–Montreal Global Biodiversity Framework. Environmental DNA (eDNA) analysis now offers a non-invasive and efficient means of biodiversity assessment, but the short amplicons (400bp) on which it currently relies carry limited information, constraining the discrimination of closely related species and the evaluation of intraspecific variation. Here we developed a long-read eDNA metabarcoding approach that resolves species and intraspecific genetic diversity simultaneously. We designed a novel universal primer set amplifying a 2–3 kbp mitochondrial region spanning cytochrome b (Cytb) to the 12S rRNA gene, and evaluated its performance at three levels: aquarium experiments using multiple lineages of medaka (*Oryzias sakaizumii* and *O. latipes*) and three-spined stickleback (*Gasterosteus aculeatus*); exhibition tanks at the Lake Biwa Museum; and field sampling in Lake Biwa. In the aquarium experiments, haplotypes recovered from rearing water were compared directly with tissue DNA from the individual fish; in the exhibition tanks and the field, species detection was compared against short-read (MiFish-U) metabarcoding. By providing simultaneous access to interspecific and intraspecific information, this approach can establish a basis for high-resolution and scalable biodiversity monitoring, opening access to ecological and genetic information that has remained largely inaccessible to conventional and short-read methods.

【P-29】

環境 DNA を用いた石川県の河川の魚類相調査 ～学校間ネットワークによる 広域調査～

Fish Fauna Survey of Rivers in Ishikawa Prefecture Using Environmental DNA: A Large-Scale Investigation via an Inter-School Network

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石川県の河川における魚類相の変化や外来種の拡大状況を把握するため、高校生ネットワークと専門機関の共同研究により、1996年の調査地点のうち、160地点で環境DNAによる調査を実施した。

種特異的解析では、アユとドジョウが県内の多数地点に分布することが示された。網羅的解析の結果、全65種を確認し、希少種の分布や特定外来生物の分布拡大が明らかになった。多変量解析等から魚類相には明確な地域性が認められた。加賀・金沢周辺は平野部河川の魚種や外来魚が多く群集構造が類似する一方、能登地域は森林や急勾配環境を反映し溪流性魚類が多くみられた。七尾湾流入河川では汽水・海産魚と淡水魚が混在し、河川ごとの環境に応じた多様な群集が構成されていることが示唆された。2026年には残り200地点で調査を行う。これにより、石川県下の河川355地点の魚類相を報告する予定である。

To assess changes in fish fauna and the spread of invasive species in Ishikawa Prefecture's rivers, a joint study by a high school network and specialized institutions conducted environmental DNA (eDNA) surveys at 160 sites previously examined in 1996.

The eDNA analysis detected 65 species in total. Species-specific assays showed that sweetfish (*Plecoglossus altivelis*) and weather loach (*Misgurnus anguillicaudatus*) are widely distributed across the prefecture. The comprehensive analysis revealed the distribution of rare species and the expansion of designated invasive alien species. Multivariate analysis demonstrated clear regional variations in fish communities: Kaga and Kanazawa featured similar community structures dominated by lowland and invasive species; Noto was characterized by mountain-stream species reflecting its forested and steep geography; and rivers draining into Nanao Bay hosted diverse communities with co-existing freshwater, brackish, and marine species.

Surveys at the remaining 200 sites are scheduled for 2026. Ultimately, this ongoing project aims to report on the fish fauna across a total of 355 river sites in Ishikawa Prefecture.

【P-30】

河川生態系における微生物相および魚類相の形成の鍵となる水質項目の探索

Exploring key water quality parameters governing the formation of microbial and fish communities in river ecosystems

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環境 DNA 分析では、微生物から魚類まで多様な分類群を同一試料から解析できる。本研究では、河川生態系における環境 DNA 分析による生物相データと水質分析データを統合解析し、微生物相および魚類相の形成の鍵となる水質項目を探索した。調査は福岡県内の 4 河川の感潮域において、1 年間にわたり毎月干潮時に実施した。魚類相は Jaccard 指数、微生物相は Bray-Curtis 指数で類似度を算出した。これらの群集構造は非計量多次元尺度構成法で比較し、さらに、R パッケージ *vegan* の *envfit* 関数を用いて各水質項目との相関を解析した。その結果、有機物および窒素化合物の指標は微生物相・魚類相の両方と有意な相関を示したことから、これらは河川生態系における両生物相の形成の鍵となる水質項目である可能性が示唆された。本研究の一部は JSPS 科研費 JP22K04390 及び JP25K01361 の助成を受けて実施した。

Environmental DNA (eDNA) analysis enables the simultaneous assessment of diverse taxonomic groups, ranging from microorganisms to fish, from a single water sample. In this study, we integrated eDNA-based biological community data with water quality data to identify the key water quality parameters shaping microbial and fish communities in river ecosystems. Monthly surveys were conducted at low tide over one year in the tidal reaches of four rivers in Fukuoka Prefecture, Japan. Community similarity was evaluated using the Jaccard index for fish communities and the Bray-Curtis index for microbial communities. Community structures were compared using non-metric multidimensional scaling (NMDS), and correlations between biological communities and water quality parameters were analyzed using the *envfit* function in the *vegan* package for R. The results showed that indicators of organic matter and nitrogen compounds were significantly correlated with both microbial and fish communities, suggesting that these water quality parameters may play key roles in shaping microbial and fish communities in river ecosystems. This study was partially supported by JSPS KAKENHI Grant Numbers JP22K04390 and JP25K01361.

【P-31】

環境 DNA による魚類バイオマス推定の検討：地点間および年度間クロスバリデーション

eDNA-based fish biomass estimation: cross-validation across sites and years

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環境 DNA 分析の主要な目標の一つは、環境試料から生物の個体数やバイオマスを推定することである。魚類では環境 DNA 量とバイオマスとの間に正の関関係が多く報告されているが、その多くは両者の相関関係を評価したものであり、推定モデルを異なる地点や年度のデータに適用した際の予測性能は、十分に検証されていない。本研究では、同所的に生息する河川性サケ科魚類 2 種を対象として、約 20 支流に設定した 28 ～ 44 地点において 3 年間、環境 DNA 量と実測バイオマスを調査した。得られたデータのうち、地点または年度単位でデータの一部を学習データとし、残りの地点または年度について、環境 DNA 量からバイオマスを推定するクロスバリデーションを実施した。これにより、環境 DNA 量に基づくバイオマス推定モデルを、未調査の地点に加え異なる年度に適用した場合の予測性能を評価し、従来の調査法を補完する手法としての有用性を検討する。

One of the primary goals of eDNA analysis is to estimate the abundance and biomass of target organisms from environmental samples. In fish, positive relationships between eDNA concentration and biomass have been widely reported. However, most studies have evaluated only the correlation between eDNA concentration and biomass, and the predictive performance of biomass estimation models when applied to different sites or years has rarely been examined. In this study, we quantified eDNA concentration and measured biomass of two sympatric stream-dwelling salmonid species over three years at 28~44 sites across approximately 20 tributaries. Using these dataset, we conducted cross-validation by using a subset of the data grouped by site or year as the training dataset and predicting biomass from eDNA concentration for the remaining sites or years. This study aims to evaluate the predictive performance of eDNA-based biomass estimation models when applied to unsurveyed sites as well as different years, and to examine their potential as a complementary approach to conventional survey methods.

【P-32】

山形県南部における環境 DNA を用いたキタノメダカ及びミナミメダカの生息域調査

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山形県に生息するキタノメダカは絶滅危惧Ⅱ類に指定されているが、山形県南部における生息域の詳細は把握されておらず、保全活動を行うのが難しいと考えた。また、山形県南部にはキタノメダカとの完全交配が可能なミナミメダカが生息しており、キタノメダカの生息域が圧迫されている可能性が考えられる。そのため、キタノメダカだけではなくミナミメダカの生息域も共に調査することで、両種の生息域の重なりについても確認することとした。本校の先行研究では、継続的に山形県南部の河川 8 地点で調査を行うことで、両種の生息域の変容を把握してきた。本研究では、昨年度よりも詳細な両種の生息域の把握を目的としてこれまでよりも調査範囲を広げ、山形県南部の河川約 15 地点で調査を行う。また、DNA 解析より、ミナミメダカの由来も明らかにしてみたい。さらに、天候や水温、pH などの環境要因などにも注意しながら、両種の生息域を明らかにしていきたい。

【P-33】

千葉県ミヤコタナゴ生息地におけるミナミメダカ関東固有系統及び国内外来系統の検出

Detection of the Kanto indigenous strain and domestically introduced strains of *Oryzias latipes* in the habitat of *Pseudorhodeus tanago* in Chiba Prefecture

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千葉県内のミヤコタナゴ生息地において、ミナミメダカ関東固有系統の残存状況および国内外来系統による遺伝的かく乱の実態を環境 DNA (eDNA) 分析により明らかにすることを目的とした。県内 6 カ所 12 地点において採水を行い、MiFish とメダカ特異的プライマー MiOlatipes を混合した eDNA メタバーコーディングを実施した。得られたシーケンスデータの処理および分類群アサインには PMiFish v3.0 を使用した。分析の結果、1 地点から関東固有系統が検出され、希少な在来系統の残存が確認された。一方で、多くの地点から国内外来系統が検出され、遺伝的かく乱の深刻さが浮き彫りとなった。また、MiFish ではミナミメダカが検出されなかった場所でも特異的プライマーでは検出され、感度の差が示された。さらに、一部の地点ではミヤコタナゴが不検出となる偽陰性の課題も示され、手法の最適化の必要性が示唆された。

This study aimed to clarify the survival status of the Kanto indigenous strain of the Japanese rice fish (*Oryzias latipes*) and the current state of genetic disturbance caused by domestically introduced strains in the habitats of the endangered bitterling (*Pseudorhodeus tanago*) in Chiba Prefecture, using environmental DNA (eDNA) analysis. Water samples were collected from 6 sites, 12 locations across the prefecture. We conducted eDNA metabarcoding using a mixture of the universal fish primers (MiFish) and *O. latipes*-specific primers (MiOlatipes). Sequence data processing and taxonomic assignment were performed using the PMiFish v3.0 pipeline. The Kanto indigenous strain was detected at one location, confirming the persistence of this rare native lineage. Conversely, domestically introduced strains were detected at multiple locations, highlighting severe genetic contamination. Methodologically, *O. latipes* was undetected by MiFish at several locations where *O. latipes* was successfully detected by the specific primers, demonstrating a clear difference in sensitivity. Furthermore, *P. tanago* was not detected at some locations despite their known occupancy, indicating a false-negative issue. These findings emphasize the necessity of optimizing sampling and analytical protocols to balance universal monitoring with high-resolution strain identification in conservation areas.

【P-34】

ロングリード環境 DNA 解析によるイワナ (*Salvelinus leucomaenis*) の遺伝的多様性評価

—紀伊半島に残存する世界最南限のイワナ属在来個体群「キリクチ」生息水域への適用—

Long-read eDNA Reveals Genetic Diversity in White-Spotted Charr (*Salvelinus leucomaenis*)

Native Haplotypes Successfully Identified in Kirikuchi Charr, the Southernmost Population of the Genus *Salvelinus*

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放流に伴う交雑はイワナ (*Salvelinus leucomaenis*) の地域固有な遺伝的多様性を損なう要因となっている。従来、在来個体群の判定には組織片からミトコンドリア DNA ハプロタイプを解析する手法が用いられてきたが、希少個体群への負荷が課題であった。そこでロングリード環境 DNA 解析を用いて 557 bp 領域の全長解析を行い、養魚場で保存していた水試料と 30 個体の組織片解析結果を比較した。その結果、既知 7 ハプロタイプをすべて検出し、高い一致性が確認された (Pearson $r=0.977$)。さらに本手法を紀伊半島に残存する世界最南限のイワナ属在来個体群「キリクチ」の生息河川へ適用した結果、上流域ではキリクチ系統のみ、下流域では非在来系統との交雑を示すハプロタイプが検出された。本手法は Nature Tech として、科学的根拠に基づく流域保全や OECM の推進への活用が期待される。

Hybridization resulting from fish stocking has threatened the regional genetic diversity of white-spotted charr (*Salvelinus leucomaenis*). Conventional assessment of native populations relies on mitochondrial DNA haplotype analysis of tissue samples; however, this approach can impose stress on rare and vulnerable populations. Recent advances in long-read sequencing technology have enabled analysis of the full 557-bp mitochondrial cytochrome *b* (*cyt b*) region, overcoming previous technical limitations in environmental DNA (eDNA)-based haplotype identification.

In this study, archived water samples collected from a fish farm were reanalyzed using long-read eDNA sequencing and compared with tissue-derived haplotype data from 30 individuals. The eDNA approach successfully detected all seven known haplotypes identified by tissue analysis, and haplotype compositions showed strong agreement between the two methods (Pearson's $r = 0.977$). Furthermore, the method was applied to rivers inhabited by the "Kirikuchi," the southernmost remnant native population of the genus *Salvelinus* in the Kii Peninsula, Japan. Only Kirikuchi-lineage haplotypes were detected at the upstream site, whereas haplotypes indicating introgression from non-native lineages were detected downstream.

These results demonstrate that long-read eDNA analysis is an effective non-invasive tool for monitoring the genetic status of rare native populations. As a Nature Tech approach supporting the realization of a Nature Positive society, this method is expected to contribute to evidence-based watershed conservation and the promotion of Other Effective Area-Based Conservation Measures (OECMs).

【P-35】

希少種保全の現場での環境 DNA の活用 ～セボシタビラ保全協議会の取り組み～

The Use of Environmental DNA for the Conservation of Rare Species: Initiatives of the Seboshitabira Conservation Council

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平成 29 年九州北部豪雨で被災した筑後川水系の A 河川では、災害復旧事業として 5 年間にわたり大規模な河川改修が実施された。本河川には国内希少野生動植物種セボシタビラが生息しており、工事と並行して生息場再生や域外保全が進められてきた。九環協では、セボシタビラとその産卵母貝であるイシガイ類の生息状況を把握するため、令和 5 年度から約 20 地点で環境 DNA 調査を継続している。さらに令和 7 年度からは定量メタバーコーディング解析を導入し、地点間・季節間での比較評価を可能とした。環境 DNA 調査により、目視や採捕では把握が困難な希少種やイシガイ類の分布・動態を効率的に把握でき、保全対策やモニタリング結果の評価に資する重要な基礎情報が得られている。今年度からは、産官学で構成する「セボシタビラ保全協議会」が設立され、環境 DNA 調査をはじめとするモニタリング結果を共有し、保全方針の検討や対策の実施に活用している。

A river in the Chikugo River basin was severely affected by the 2017 Northern Kyushu Heavy Rain Disaster, and large-scale river restoration works were conducted over a five-year period as part of the disaster recovery project. The river is inhabited by *Acheilognathus tabira nakamurae* (Seboshitabira), a nationally protected endangered fish species in Japan. Habitat restoration and ex situ conservation have therefore been implemented in parallel with the construction works. Since 2023, we have conducted regular environmental DNA (eDNA) surveys at approximately 20 sites to monitor the distribution of Seboshitabira and unionid mussels, which serve as spawning hosts for bitterlings. In 2025, quantitative metabarcoding was introduced, enabling comparisons among sites and seasons. eDNA surveys have greatly improved the detection of Seboshitabira and unionid mussels, which are difficult to monitor using conventional visual surveys or capture methods, and have provided essential baseline information for evaluating conservation measures. In 2026, the Seboshitabira Conservation Council was established through collaboration among industry, government, and academia. The council integrates eDNA-based monitoring into adaptive conservation management by sharing monitoring data among stakeholders and using the results to guide conservation planning and management actions.

【P-36】

環境 DNA を用いた知床半島におけるアメマス (*Salvelinus leucomaenis*) ミトコンドリア DNA ハプロタイプの分布解析

Environmental DNA reveals a white-spotted charr (*Salvelinus leucomaenis*) mitochondrial DNA haplotype previously unreported from the Shiretoko Peninsula

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知床半島のオショロコマ集団では、過去の遺伝子浸透によりアメマス由来のミトコンドリア DNA (mtDNA) を保有する個体の存在が知られている。近年、この mtDNA は知床半島近傍のアメマス集団で見られるものとは異なる単一のハプロタイプに限られることが示唆されており、古い種間交雑に由来すると考えられている。本研究では、イワナ属の mtDNA ハプロタイプを識別できる環境 DNA 検出系を開発し、知床半島内 50 河川の水試料に適用した。その結果、これまで知床半島内では報告されていなかった、アメマスの mtDNA ハプロタイプを、調査対象 50 河川のうち 4 河川で検出した。この新たに検出されたハプロタイプの起源を解明することは、アメマスの分布史やオショロコマとの歴史的な種間交雑に対する理解を深めるとともに、今後、気候変動がアメマスの分布に及ぼす影響を解明するための基盤となることが期待される。

Southern Asian Dolly Varden (*Salvelinus curilus*) populations on the Shiretoko Peninsula are known to harbor mitochondrial DNA (mtDNA) derived from white-spotted charr (*Salvelinus leucomaenis*) through historical introgression. Recent studies have suggested that this introgressed mtDNA is restricted to a single haplotype distinct from those found in nearby *S. leucomaenis* populations, indicating that it originated from ancient interspecific hybridization. In this study, we developed an environmental DNA (eDNA) assay capable of discriminating multiple *Salvelinus* mtDNA haplotypes and applied it to water samples collected from 50 rivers across the Shiretoko Peninsula. Our analysis detected a mtDNA haplotype not previously reported from the Shiretoko Peninsula in four of the 50 surveyed rivers. Elucidating the origin of this newly detected haplotype will improve our understanding of the distributional history of *S. leucomaenis* and its historical hybridization with *S. curilus*, while also providing a basis for future studies investigating the influence of climate change on the distribution of *S. leucomaenis*.

【P-37】

環境 DNA を用いた江の川土師ダム下流におけるアユの時空間分布の把握

Estimating the Spatiotemporal Distribution of Ayu (*Plecoglossus altivelis*) Downstream of the Haji Dam in the Gonokawa River Using Environmental DNA

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近年、全国で重要種であるアユ (*Plecoglossus altivelis*) の漁獲量が減少傾向にあり、効率的な生息地の把握・保護が求められている。しかし、複数年にわたるアユの動態を定量的に把握した研究は限定的である。そこで本研究では、2021 年から 2022 年の 7,8,10 月において、江の川土師ダム下流 (土師ダムから下流約 40km) の主要な 16 の支川および支川合流前の本川において環境 DNA 分析を実施し、アユの空間分布の変遷を調査した。その結果、本川では遡上行動によって上流側が主要な生息場となっていることや、出水によって一時的に個体群が下流側に移動する可能性が示された。また支川では、出水・降下行動に伴う個体群の減少が定量的に捉えられた。本結果は、保全優先度の高い支川の特定や、好適な生息環境の評価・保全策への活用が期待される。

In recent years, catches of ayu (*Plecoglossus altivelis*), a species of importance, have been declining across Japan. Therefore, effective habitat identification and conservation of ayu are required. Studies quantitatively tracking the multi-year dynamics of ayu are still limited. In this study, we investigated the spatiotemporal shifts in ayu distribution downstream of the Haji Dam in the Gonokawa River. Environmental DNA (eDNA) analysis was conducted in July, August, and October of 2021 and 2022 across 16 major tributaries and the mainstem (the upstream of tributary junctions) along an approximately 40 km reach downstream of the dam. The results indicated that, in the mainstem, the upstream section serves as the primary habitat due to the upstream migration, and that flood events could cause the population to temporarily shift downstream. Furthermore, in the tributaries, the population declines were associated with flood events, and the downstream migration were quantitatively observed. These findings are expected to contribute to identifying high-priority tributaries for conservation, evaluating suitable habitat conditions for ayu, and developing effective conservation strategies.

【P-38】

宍道湖におけるニホンウナギの時空間的分布動態とその要因の解明

Spatiotemporal distribution patterns of the Japanese eel (*Anguilla japonica*) in Lake Shinji and their environmental drivers

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ニホンウナギは日本において古くから利用されてきた重要な水産資源である。しかし近年個体数が減少し、IUCN レッドリストでは絶滅危惧 (EN)、国内の環境省レッドリストでは絶滅危惧 I B 類にリストされている。本種は夜行性であり、日中は岩の隙間や河畔植生の下を隠れ場所として利用するため、従来の目視調査や捕獲による調査は時間と労力を要する。そこで本研究では、ウナギの短鎖散在反復配列 (SINE) を標的としたプライマープローブを用いた環境 DNA 分析により、宍道湖において 2016 年から 2025 年までの 10 年間にわたり継続的に採取された水試料を分析し、ニホンウナギの時空間的な分布変動を明らかにする。さらに、その結果を時間的および空間的に比較することにより、ニホンウナギの分布動態を規定する要因を明らかにすることを目的とする。

The Japanese eel (*Anguilla japonica*) has long been an important fishery resource in Japan. However, its population has declined markedly in recent decades, and the species is currently classified as Endangered (EN) on the IUCN Red List and as Endangered IB (EN) on the Ministry of the Environment Red List of Japan. Because the Japanese eel is nocturnal and typically uses rock crevices and areas beneath riparian vegetation as daytime refuges, conventional monitoring methods, such as visual surveys and capture-based surveys, require considerable time and effort. In this study, we used environmental DNA (eDNA) analysis with a species-specific primer–probe set targeting the short interspersed nuclear element (SINE) of the Japanese eel to analyze water samples collected continuously from Lake Shinji over a 10-year period (2016–2025) to clarify the spatiotemporal variation in the distribution of the Japanese eel. Furthermore, by comparing the results across temporal and spatial scales, we aimed to identify the factors determining the spatiotemporal distribution dynamics of the Japanese eel.

Repeated eDNA Monitoring Reveals Spatial Variation in Amphibian Occurrence and Assemblage Composition

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Repeated eDNA sampling at fixed sites can reveal spatial differences that persist across survey periods. Using single-species and multispecies case studies in South Korea, we examined whether repeated eDNA surveys captured spatial and temporal variation in amphibian occurrence and assemblage composition. For the single-species study, monthly eDNA sampling and visual larval surveys for *Onychodactylus sillanus* were conducted from March to November 2024 at 17 sites spaced 50m apart along a headwater tributary on Mount Geumjeong, yielding 216 water samples for qPCR analysis. For the multispecies study, amphibian metabarcoding was performed on 85 water samples collected during five surveys from March to December 2025 at 17 fixed sites representing five habitat types in Pyeongdume Wetland. In the single-species analysis, eDNA detection frequency across surveys was positively associated with cumulative larval observations across sampling sites ($r = 0.487$, $p = 0.048$), indicating that repeated eDNA monitoring can capture spatial variation in occurrence within the stream. In the multispecies analysis, six amphibian taxa were detected, and taxonomic composition differed significantly among habitat types after accounting for survey month (PERMANOVA, $R^2 = 0.222$, $p = 0.027$). These habitat-specific patterns were broadly consistent with the known habitat associations of the detected taxa. Across both systems, repeated sampling revealed spatial differences supported by larval observations in the stream and known habitat associations in the wetland. Repeated eDNA monitoring can therefore move beyond presence records by revealing spatial differentiation in amphibian occurrence and assemblage composition.

Torrent in a Bottle: Developing Environmental DNA Protocols for Monitoring Endangered Torrent Salamanders

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The torrent salamanders (Rhyacotritonidae) are a family of headwater-obligate species endemic to the Pacific Northwest. Two species (*R. kezeri* and *R. cascadae*) are being considered as candidates for listing under the United States Federal Endangered Species Act (ESA) and will be a conservation focus in the decades to come. Monitoring can be challenging, as their ability to utilize the hyporheic zone, where subsurface stream flow mixes with groundwater, as well as their unique life history make them difficult to survey by traditional methods. Environmental DNA (eDNA), a non-invasive method of detecting aquatic species, could be a valuable survey and detection tool for managers and researchers studying these elusive species. We developed species specific primers for both *R. kezeri* and *R. cascadae*, pairing physical surveys with eDNA surveys to assess effectiveness in detection within sites across their range. We successfully detected both species at multiple sites, demonstrating the usefulness of eDNA surveys as a cost-effective alternative to traditional surveys which often result in significant microhabitat disturbance. Using Bayesian hierarchical models, we examined the relationship between observed salamander abundance with eDNA quantification and detection probabilities. We found that eDNA was a much more effective tool for *R. kezeri*, although we also found evidence that lower abundances in *R. cascadae* may account for the reduced efficiency of this survey tool. Using our survey data, we demonstrate how eDNA is particularly useful for *R. kezeri* given high detection rates across sites, whereas managers may need to utilize multiple sampling techniques with *R. cascadae* to consistently detect occupancy with high confidence.

【P-41】

セトウチサンショウウオの生息環境改善に向けた堆積物除去効果の評価

Evaluating pond sediment removal as a habitat restoration strategy for *Hynobius setouchi*

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里山のため池はセトウチサンショウウオにとって重要な繁殖地であるが、近年は耕作放棄に伴う環境悪化が懸念されている。これまでの調査から、利用されなくなったため池では夏季に本種の環境 DNA 検出率が低く、山林化に伴う溶存酸素濃度の低下が一因であると示唆された。そこで本研究では、生息環境の改善を目的に堆積物除去を実施し、その効果を環境 DNA 分析で評価した。兵庫県神戸市のため池 10 地点で 2023 年 4 月～2025 年 9 月、2026 年 1 月～8 月に月 1 回採水調査を行うのと並行し、2024 年 12 月にひとつのため池で堆積物除去を実施し、2025 年 12 月に別のため池で同様に実施した。その結果、両池とも堆積物除去後に環境 DNA 検出率が増加し、採捕調査でも幼生が確認された。これらの結果は、堆積物除去が本種の繁殖環境改善に有効である可能性を示すとともに、環境 DNA 分析が管理効果を評価する有用なツールとなることを示唆した。

Hynobius setouchi relies on irrigation ponds in Satoyama landscapes as important breeding habitats. However, habitat degradation associated with agricultural abandonment has become a growing conservation concern. Previous surveys have shown that eDNA detection rates of this species are low during summer in abandoned ponds, suggesting that reduced dissolved oxygen concentrations associated with vegetation succession toward forested conditions is a contributing factor. In this study, we removed accumulated bottom sediment, including mud and leaf litter, to improve habitat conditions and evaluated the effectiveness of this management action using eDNA analysis. Water sampling was conducted monthly at 10 irrigation ponds in Kobe City, Hyogo Prefecture, from April 2023 to September 2025 and from January to August 2026. In parallel with these surveys, sediment removal was carried out at a pond in December 2024 and at another pond in December 2025. Following sediment removal, eDNA detection rates increased in both ponds, and larvae were also confirmed through capture surveys. These results suggest that sediment removal may be effective in improving habitat conditions for *H. setouchi* and that eDNA analysis can serve as a useful tool for evaluating the effectiveness of habitat management.

【P-42】

山形県置賜地域におけるサンショウウオの環境 DNA を用いた生息域調査

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準絶滅危惧種に指定されているトウホクサンショウウオは、センザンサンショウウオ・バンエツサンショウウオ・トウホクサンショウウオの3種に再分類された（藤原 2024）。3種は遺伝子と形態の違いにより分類されたが、その生息域や生息条件の詳細は明らかとなっていない。山形県置賜地域にはセンザン・バンエツの境界があると言われており、早急な生息域の調査と保全が必要である。そこで現状を把握するため、個体や生息地を損なわない環境 DNA による生息域調査を行うこととした。本校の先行研究（2025）において、センザン・バンエツの mtDNA の Cytb 領域全長の塩基配列解析を行い、プライマーを設計したが、特異性は確認できなかった。本年度は、制限酵素を用いた手法によるセンザンサンショウウオ・バンエツサンショウウオの2種の識別方法を研究している。そして、環境 DNA 調査により2種の生息域を明らかにし保全につなげたい。

【P-43】

アマガエル属 2 種の分布境界解明に向けて

Toward delineating distribution boundary between two *Dryophytes* frog species

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近年の研究により、本州に広く分布するアマガエル類は、近畿地方を境に東部に分布するヒガシニホンアマガエル *Dryophytes leopardus* と西部に分布するニホンアマガエル *D. japonicus* の 2 種に分かれることが判明した。しかし、両種を形態のみで識別することは困難であり、近畿地方周辺では現在でも分布状況が不明な地域が多い。両種の詳細な分布を把握することは保安全管理に資するだけでなく、生物の歴史的な分散を規定してきた地理的障壁を理解する上でも重要である。そこで本研究では、環境 DNA から両種を同時に検出可能なマルチプレックス PCR 検出系を開発した。また野外適用として、両種の生息状況が不明な福井県および三重県で本検出系を用いた調査を行った。本検出系は、捕獲を伴わずに広域的な分布調査を可能にすることから、両種の分布境界や接触帯の解明に有用であると期待される。

Recent studies have revealed that the tree frogs widely distributed across Honshu consist of two species: *Dryophytes leopardus*, distributed in eastern Japan, and *D. japonicus*, distributed in western Japan, with their boundary located around the Kinki region. However, distinguishing these species based solely on morphology is difficult, and many areas around the Kinki region remain poorly characterized with respect to species distribution. Clarifying their detailed distributions is important not only for conservation and management but also for understanding the geographic barriers that have shaped the historical dispersal of organisms. In this study, we developed a multiplex PCR-based eDNA assay capable of simultaneously detecting both species. As a field application, we also conducted a survey in Fukui and Mie Prefecture, where the distributional status of the two species remains unclear. Because this assay enables broad-scale distribution surveys without the need for capture, it is expected to be a useful tool for elucidating the distribution boundary and contact zone between the two species.

【P-44】

創出したビオトープにおける長期モニタリングに向けた環境 DNA の適用検討

Assessing the Application of Environmental DNA for Long-term Monitoring of a Created Biotope

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開発に伴う環境保全対策として創出したビオトープでは、建設工事竣工後 10 年間にわたり、生物の定着状況や生息環境の変化を把握するためのモニタリングを継続してきた。本研究では、竣工 10 年目調査として、従来の生物採捕調査に加え、MiFish（魚類）および Amph16S（両生類）を用いた環境 DNA 分析を導入し、長期モニタリングへの適用性について検討した。現地調査では魚類および両生類を対象とした採捕調査を実施し、カエル類やイモリ類の生息を確認するとともに、ドジョウ類を採捕した。環境 DNA 分析による検出結果と従来調査結果との比較を行うほか、採捕したドジョウについては、地域在来系統の確認および交雑・外来系統の混入状況を評価するため、配列情報を用いた系統解析を進めている。これらの結果を踏まえ、創出したビオトープにおける長期モニタリングへの環境 DNA の適用可能性について考察する。

A biotope created as an environmental conservation measure associated with a development project has been monitored for ten years after construction to evaluate biological establishment and changes in habitat conditions. As part of the 10-year post-construction survey, environmental DNA (eDNA) analysis using MiFish and Amph16S was introduced in addition to conventional biological capture surveys to assess its applicability for long-term ecological monitoring. Field surveys targeted fish and amphibians, confirming the presence of frogs and newts, while loaches were collected for further analysis. The eDNA results were compared with those obtained from conventional surveys. In addition, phylogenetic analyses based on MiFish sequence data are being conducted to evaluate the regional native lineage of the collected loaches and to assess the possible occurrence of hybrid or non-native lineages. Based on these findings, we discuss the applicability of eDNA for long-term monitoring of created biotopes.

【P-45】

定川及び赤井堀排水路における水生生物調査

Aquatic Organism Survey in the Joga River and Akaibori Drainage Channel

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私たちは定川及び赤井堀排水路の水生生物調査を行いました。定川とは宮城県石巻西高校の近くを流れる二級河川です。宮城県が実施した平成 27 年度の調査ではウナギやタナゴ、ドジョウなどが生息していることが分かっています。私たちは、定川と隣接する赤井堀排水路（用水路含む）に生息している生物を詳しく調べたいと考え、石巻専修大学、東北大学の方々に協力していただき、採集調査と環境 DNA 調査を実施することにしました。

これまでに 6 月 22 日に 1 回目、7 月 27 日に 2 回目の水生生物調査を行いました。8 月 6 日には環境 DNA 調査を行う予定です。調査結果は 12 月以降に判明する予定です。

今回は、2 回の生物調査の結果について発表します。個体数については外来種が在来種よりも圧倒的に多く、特にアメリカザリガニとカラドジョウが突出して多いことが分かりました。しかし、環境省で指定された準絶滅危惧種と思われる生物も 2 種見つかりました。

Release Dynamics and Sample-Type-Specific Detection of *Trigomphus melampus* eDNA

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Trigomphus melampus (Odonata: Gomphidae) is a dragonfly endemic to Japan that inhabits vegetated ponds and wetlands. In Nara Prefecture, it is designated as a protected species, and its distribution remains poorly understood. Environmental DNA (eDNA) analysis may be useful for exploring potential habitats as a non-invasive method that does not require collecting individuals. However, in low-density aquatic insects, eDNA concentrations may be low and spatially heterogeneous, so field detection may depend on sampling conditions. Information on eDNA release dynamics and appropriate sample types for detecting this species is still lacking. In this study, using species-specific primers and a probe, we examined (1) eDNA release dynamics from a single larva maintained in a 1-L container over 48 h before and 24 h after molting, and (2) detection probabilities among three sample types (water collected before sediment disturbance, and supernatant and sediment collected after disturbance) collected from a pond where *T. melampus* was present. Preliminary results indicated that eDNA concentrations increased substantially after molting, and detection probability differed among sample types, with sediment-derived samples showing higher detection success than undisturbed surface water. These findings provide practical guidance for designing field eDNA surveys of *T. melampus* and other rare aquatic insects with low detectability. We further present preliminary results from ongoing eDNA-based field surveys in Nara Prefecture.

【P-47】

アオハダトンボの検出系の開発と野外適用

Development of eDNA assay for *Calopteryx japonica* and its field application

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アオハダトンボ *Calopteryx japonica* は、水生植物が繁茂する平地や丘陵地の清流域に生息するが、近年は河川環境の悪化により、一部地域で個体数の減少が報告されている。これに加えて、幼虫の時期では近縁種であるハグロトンボとの形態的判別が困難であることから、保全に向けては、非侵襲的かつ近縁種と識別可能なモニタリング手法の確立が求められる。そこで本研究では、アオハダトンボを対象とした 16S rRNA 領域の種特異的環境 DNA 検出系を開発し、兵庫県丹波篠山市の篠山川での野外適用を試みた。しかし、野外試料では検出が不安定であった。この課題に対し、検出感度の向上を目的として、16S rRNA 領域に加え、複数領域を併用するマルチマーカの有効性についても検討する。これらを通じて、本種の生息状況の把握および保全モニタリングへの応用可能性を評価する予定である。

Calopteryx japonica inhabits clear streams in flat grounds and hilly areas where aquatic vegetation is abundant, but in recent years its population has declined in some regions due to the deterioration of river environments. In addition, larvae are difficult to distinguish morphologically from those of the closely related species, *C. atrata* so there is a need to establish a non-invasive monitoring method that can simultaneously distinguish between the two species for conservation purposes. In this study, we developed a species-specific eDNA detection assay targeting the 16S rRNA region for *C. japonica* and attempted to apply it to field samples collected from Sasayama River, Hyogo, Japan. However, detection was unstable in field samples. Therefore, we plan to evaluate the effectiveness of a multi-marker approach that combines multiple genetic regions in addition to the 16S rRNA marker to improve detection sensitivity. Through these approaches, we assess the applicability of this method for monitoring the distribution of *C. japonica* and for its conservation.

【P-48】

ヘイケボタルの飛翔成虫および発光幼虫の個体数および湿地内分布と環境 DNA 濃度との関連性の検証

Assessing the Relationship between Environmental DNA Concentration and the Abundance and Within-Wetland Distribution of Flying Adults and Luminescent Larvae in the Firefly *Aquatica lateralis*

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神奈川県横浜市内の谷戸に整備した約 300 m²の湿地において、2024 年度からヘイケボタルの個体数調査と環境 DNA 分析を継続している。個体数調査では、ルートセンサスにより上陸直前期の水中の発光幼虫と飛翔成虫の個体数を計測し、発光幼虫は分布を記録した。環境 DNA 分析では、湿地の下流から上流までの 4 地点で採水し、種特異的解析により DNA 濃度を定量した。

多年度データを比較した結果、幼虫期の環境 DNA 濃度と飛翔成虫の個体数の増減との間に関連が認められた。また、全ての調査年度で上陸直前期の発光幼虫が上流側に多く分布し、同地点で高い DNA 濃度が検出された。以上から、環境 DNA 分析はヘイケボタルの個体数変動および湿地内の分布を把握する手法として有効であることが示唆された。

We have conducted annual surveys of the aquatic firefly *Aquatica lateralis* (= *Luciola lateralis*) in an approximately 300 m² constructed wetland in a yato (a narrow headwater valley typical of the Kanto region) in Yokohama, Kanagawa Prefecture, Japan, since fiscal year 2024. During route censuses, we counted flying adults and mature luminescent larvae immediately before they left the water to pupate on land, and we recorded the spatial distribution of the larvae. For environmental DNA (eDNA) analysis, we collected water samples at four sites along a downstream-to-upstream gradient within the wetland and quantified eDNA concentrations using a species-specific qPCR assay. Comparison of data across survey years indicated an association between eDNA concentrations during the larval stage and year-to-year changes in the abundance of flying adults. In every survey year, mature larvae were more abundant in the upstream section than in the downstream section, and eDNA concentrations were correspondingly higher there. These findings suggest that eDNA analysis is a useful approach for assessing both year-to-year changes in abundance and the within-wetland spatial distribution of *A. lateralis*.

【P-49】

核 ITS 領域を対象とした eDNA 種内変異解析による水生昆虫の個体群間交流推定

Inferring population connectivity of aquatic insects using eDNA-based intraspecific analysis targeting the nuclear ITS region

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成虫期に陸域を飛翔する水生昆虫の移動分散や個体群間交流には、未解明な点が多い。また、移動分散様式に性差がある場合には、ミトコンドリアマーカを対象とした調査のみでは遺伝子流動の実態を十分に評価できない可能性がある。そこで本研究では、カゲロウ 2 種（エルモンヒラタカゲロウおよびマツムラヒラタカゲロウ）とトビケラ 2 種（ヒゲナガカワトビケラおよびチャバネヒゲナガカワトビケラ）を対象に、核 ITS 領域を用いた環境 DNA 種内変異解析法を開発し、奈良県吉野川の上中流域において広域調査を実施した。核 ITS 領域とミトコンドリア COI 領域から得られた遺伝構造を比較解析することにより、性差を考慮した移動分散パターンや個体群間交流の評価を試みた結果を報告する。

Understanding dispersal and population connectivity is essential for ecological research and conservation of aquatic insects. However, direct observation of adult flight is challenging, leaving dispersal and connectivity poorly understood. Sex-specific dispersal may also limit the ability of mitochondrial markers alone to capture patterns of gene flow. We developed eDNA-based intraspecific analyses targeting the nuclear ITS region in two mayfly species (*Epeorus latifolium* and *E. l-nigrus*) and two caddisfly species (*Stenopsyche marmorata* and *S. sauteri*), and conducted a broad survey across the upper and middle reaches of the Yoshino River, Nara Prefecture, Japan. By comparing population genetic structures inferred from the nuclear ITS and mitochondrial COI regions, we evaluated dispersal and population connectivity while accounting for potential sex differences.

【P-50】

Koro-rin 法を用いた外来種スクリーニングの可能性

Application of the Koro-rin Method to Invasive Species Screening

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陸域の環境 DNA 調査では、DNA が局所的に存在するため、広域かつ効率的なサンプリングが課題となる。本研究では、陸域環境 DNA の回収技術である Koro-rin 法を用い、アルゼンチンアリを対象とした環境 DNA の検出を試みた。各地で採取したサンプルを解析した結果、アルゼンチンアリ DNA が検出される地点が確認された。また、一部の地点では実個体も確認されており、Koro-rin 法による環境 DNA 検出がアルゼンチンアリの生息地点候補を抽出するスクリーニング手法としての有用性が示唆された。一方で、環境 DNA の由来や空間的な広がりを含め、検出結果の解釈には検討すべき課題も残されている。本発表では、これらの結果を踏まえ、Koro-rin 法の外来種モニタリングへの適用可能性と今後の展望について議論する。

Terrestrial environmental DNA (eDNA) surveys face challenges in achieving efficient large-scale sampling because eDNA is distributed locally. In this study, we attempted to detect eDNA of the invasive Argentine ant (*Linepithema humile*) using the Koro-rin method, a terrestrial eDNA collection technique. Analysis of samples collected from multiple sites confirmed the presence of Argentine ant eDNA at several locations. Furthermore, live Argentine ant individuals were also observed at some of these sites, suggesting that eDNA detection using the Koro-rin method may serve as a screening approach for identifying candidate locations for Argentine ants occurrence. However, challenges remain in interpreting detection results, including the origin and spatial extent of the detected eDNA. Based on these findings, we discuss the use of the Koro-rin method for invasive species monitoring and future directions.

【P-51】

簡易地表面サンプリングによるキョン eDNA 量と生息地利用の関係評価に挑戦

Assessing the relationship between muntjac eDNA quantity from simple ground surface sampling and habitat use

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陸上動物では eDNA 量と生息密度や実際の利用状況との関係性を評価した研究はごくわずかである。環境 DNA によって陸上動物の生息有無だけではなく、利用強度等の評価ができれば、野生動物の生息調査にイノベーションを起こせる。そこで私たちは特定外来生物であるキョン由来の DNA の量とキョンの利用状況との関係評価に挑んだ。eDNA は簡易な地表面サンプリングをし、キョンの種特異プライマーを用いてリアルタイム PCR で分析した。一部の調査地では複数のサンプリング方法（環境水、葉面、水を張ったバケツの設置）での比較も実施した。キョンの利用状況は自動撮影カメラの撮影画像から評価した。地表面サンプリングでは 22 カ所中 21 カ所でキョンの DNA が検出され、複数手法を比較した 3 カ所のうち、全地域で検出したのは地表面サンプリングのみであった。利用状況との関係性については絶賛分析中であり、会場での発表を待たれたい!!

Very few studies have assessed the relationship between eDNA levels and actual habitat use in terrestrial animals. If eDNA could be used not only to detect the presence of terrestrial animals but also to assess factors such as the intensity of their use, this could revolutionize population survey methods. We therefore set out to evaluate the relationship between DNA derived from the muntjac *Muntiacus reevesi* - a invasive alien species- and its population density and habitat use. eDNA was collected via simple ground-surface sampling and analyzed using real-time PCR with species-specific primers for the muntjac. At some survey sites, comparisons were also carried out using multiple sampling methods (river water, leaf surfaces, and buckets filled with water). Muntjac usages were assessed based on data such as images captured by camera traps. In the surface sampling, muntjac DNA was detected at 21 out of 22 sites. Of the three sites where multiple methods were compared, only ground-surface sampling detected the target in all sites. Analysis of the relationship with habitat use is currently underway; please look forward to the presentation at the conference!!

【P-52】

糞便由来 DNA を用いた野生哺乳類の種同定とツキノワグマ識別への応用

Species identification of wild mammals using fecal DNA: application to Asiatic black bear detection

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近年、糞便由来 DNA を用いた非侵襲的な野生動物調査が広く利用されている。本研究では、野生哺乳類の種同定およびツキノワグマ識別への応用を目的として、既存プライマーの性能評価、ツキノワグマ種特異的プライマーの開発、ならびに環境要因が糞便 DNA の残存量に与える影響の解析を行った。その結果、MiMammal-U/B は幅広い哺乳類種を高精度に同定できた。また、開発したツキノワグマ種特異的プライマーは高い特異性を示した。さらに、日光と降雨の相互作用が DNA 残存量に影響する可能性が示された。以上より、糞便 DNA 解析はツキノワグマを含む野生哺乳類調査に有効であることが示唆された。今後は、開発したツキノワグマ種特異的プライマーの環境 DNA 試料への適用性を評価し、野外におけるツキノワグマの DNA 検出への応用を目指す。

Non-invasive wildlife surveys using fecal DNA have become increasingly common in recent years. This study aimed to evaluate species identification methods for wild mammals and their application to the detection of the Asiatic black bear (*Ursus thibetanus japonicus*). We assessed the performance of existing primer sets, developed an Asiatic black bear-specific primers, and investigated the effects of environmental factors on the persistence of fecal DNA. MiMammal-U/B enabled accurate identification across a wide range of mammalian species. In addition, the newly developed Asiatic black bear-specific primers showed high specificity. Furthermore, interactions between sunlight and rainfall were found to potentially influence DNA persistence. These results suggest that fecal DNA analysis is an effective tool for surveying wild mammals, including the Asiatic black bear. Future studies will evaluate the applicability of the newly developed Asiatic black bear-specific primer to eDNA samples and its potential for field-based detection of Asiatic black bears.

【P-53】

里山林における Koro-rin 法を用いた鳥類調査手法の検証：観察調査との比較

Evaluation of bird survey with Koro-rin method in *Satoyama* forests: Comparison with visual surveys

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高い移動能力から気候変動や資源量変化を迅速に反映する鳥類は、生態系の指標生物として重要である。鳥類モニタリングは、生態系や環境の変化を把握するために有効であるが、従来の鳥類調査では観察されやすい種に記録が偏ることや、短期間に多地点を調査することが困難であるといった課題があった。本研究では、自然共生サイトである神戸市北区の里山（山林および棚田景観）を対象に、陸域環境 DNA のサンプリング法である Koro-rin 法を用い、標準的な鳥類調査法である観察法と比較した。2026 年 4 月に 10 地点で実施した調査では、環境 DNA で 22 種、観察で 21 種を検出し、両手法で検出した種は 9 種であった。この結果は、環境 DNA 手法が観察法では検出が困難な種を補完し、より実態に即したモニタリングを実現し得る可能性を示している。発表では 2026 年 5 月と 7 月の調査結果を加え、より詳細な議論を行う予定である。

Birds, which can rapidly reflect changes in climate and resource availability due to their high mobility, are important indicator species for ecosystems. While bird monitoring is an effective method for understanding changes in ecosystems and the environment, conventional bird surveys have several limitations, such as a bias toward species that are easy to observe and the difficulty of surveying multiple sites within a short period. In this study, we applied the Koro-rin method, a terrestrial eDNA sampling technique, in a *Satoyama* landscape (comprising forests and terraced rice fields) located in Kita Ward, Kobe City, Japan, which has been designated as a "Nationally Certified Sustainably Managed Natural Sites". We compared the performance of this method with that of conventional visual bird surveys. In a survey conducted at 10 sites in April 2026, 22 species were detected using eDNA and 21 species through visual observation; 9 species were detected by both methods. These results indicate that the eDNA method can complement conventional visual observation by detecting species that are difficult to detect through visual observation, potentially enabling monitoring that more accurately reflects the actual situation. In the presentation, we plan to include survey results from May and July 2026 to provide a more detailed discussion.

【P-54】

ホシガレイ放流種苗を捕食するカワウの採餌活動の環境 DNA モニタリング

Environmental DNA monitoring of foraging activity of great cormorants on released spotted halibut juveniles

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福島県松川浦では、ホシガレイ *Verasper variegatus* 種苗が継続して放流されており、先行研究にて放流後にカワウ *Phalacrocorax carbo* による捕食が目視で観察された。放流によるカワウの採餌行動増加を観測するため、本研究では環境 DNA 技術を応用した。

まず、国内 *Phalacrocorax* 属に特異的なプライマー・プローブを開発した。また、2026 年 6 月に放流地点にて 1 日 4 回 (8・12・16・20 時)、1 週間 (放流前 2 日、当日、後 4 日) の採水を行い、リアルタイム定量 PCR により DNA 濃度を測定した。その結果、放流後は検出数が増加し、検出頻度は採餌行動の増加を表すことが示唆された。一方で、定量結果から本研究の方法では DNA 収量が少なく、手法には改善の余地が残る。今後は同時期の映像記録に基づく飛来数・潜水数と本研究で得た DNA 濃度・検出頻度との比較を行う予定である。

In Matsukawa-ura Lagoon, Fukushima Prefecture, juveniles of spotted halibut *Verasper variegatus* have been continually released to enhance the stock, and previous studies have shown that juveniles are preyed on by great cormorants *Phalacrocorax carbo* around the release site after the release. In this study, we applied an environmental DNA method to monitor increases of cormorant foraging activity.

First, we developed Japanese *Phalacrocorax* specific primers and a probe. Water samples were collected four times (at 8:00, 12:00, 16:00, 20:00) per day over a 7-days period (2 days before, on the day of, and 4 days after release). DNA concentrations were measured using real-time quantitative PCR. As a result, the number of positive detections increased after release, suggesting that detection frequency can be an indicator of the cormorant foraging activity. On the other hand, quantitative analyses showed low DNA yields in this study. In future work, we will compare the number of landings on the water and dives of cormorants recorded by a video camera during the same period with DNA concentrations and detection frequencies obtained in this study.

【P-55】

地上環境 DNA を用いたミツバチヘギイタダニの検出

Detecting *Varroa destructor* in honey bee hives by using terrestrial environmental DNA

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ミツバチヘギイタダニ *Varroa destructor* は主にセイヨウミツバチ *Apis mellifera* に寄生し、脂肪体の摂食やチブレバネウイルス (DWV) などのウイルス感染によってコロニー崩壊を引き起こす外部寄生性のダニである。ミツバチの巣箱内におけるヘギイタダニを効率的に駆除するためには、寄生の早期発見とそれに続く薬剤防除が重要である。しかし、現在寄生の検出に一般的に用いられているシュガーロール法は、ミツバチ成虫を傷つけてしまうリスクがある。そこで本研究では、巣箱の環境 DNA を用いてヘギイタダニの寄生の有無を判別する手法の開発を目指す。

Varroa destructor is an ectoparasitic mite that primarily infests the western honey bee *Apis mellifera*. *Varroa destructor* causes colony collapses through consumption of the host's fat body and the transmission of viruses such as *Deformed wing virus*. Early detection of the infestation followed by chemical control is crucial for efficient control of *V. destructor* within honey bee hives. However, the "sugar roll" method, the current standard technique for detecting *V. destructor* infestations has a risk of injuring honey bees. Therefore, this study aims to develop a method for judging the presence or absence of the infestation in honey bee hives using environmental DNA (eDNA).

【P-56】

佐渡演習林内の流域における河川水・堆積物・表土中の植物 eDNA 組成の比較

Comparison of Plant Taxonomic Information Derived from eDNA Metabarcoding of Stream Water, Sediment, and Topsoil in a Cool-Temperate Watershed on Sado Island

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eDNA 解析は、生物多様性調査の手法として普及しつつあるが、陸上植物を対象とした応用例は比較的少なく、異なる環境試料から得られる情報を比較した知見も限られている。本研究では、新潟大学佐渡演習林内の大倉川上流部で、同一流域内から採取した河川水、河川堆積物および森林表土を対象に、ITS2 領域を用いた植物 DNA メタバーコーディング解析を行う。河川水は 2025 年 4 ～ 11 月に月 1 回（計 8 回）、各回 2 L を 3 反復で採取し、河川堆積物は 2024 年に 2 回採取した。森林表土は局地性を考慮し、2025 年 11 月に流域内 13 地点から採取した。各試料から検出された植物分類群の組成、多様性および主要植生との対応を比較し、河川水については季節変動も評価する。これらを通じて、各環境試料から得られる植物 eDNA 情報の特徴を整理し、植生モニタリングへの応用可能性について考察する。

eDNA metabarcoding is increasingly used as a tool for biodiversity surveys; however, its application to terrestrial plants remains relatively limited, and few studies have compared the information obtained from different types of environmental samples. In this study, plant DNA metabarcoding targeting the ITS2 region was conducted using stream water, stream sediments, and forest topsoil collected within the same watershed in the upper reaches of the Okura River in the Sado Experimental Forest of Niigata University. Stream water was collected monthly from April to November 2025 (eight sampling occasions), with three replicate 2-L samples collected on each occasion. Stream sediments were collected twice in 2024. To account for spatial heterogeneity, forest topsoil was collected from 13 locations within the watershed in November 2025. Plant taxonomic composition, diversity, and correspondence between detected taxa and the dominant local vegetation are compared among sample types, and seasonal variation is also evaluated for stream water. Through these comparisons, we evaluate the characteristics of plant eDNA information obtained from each type of environmental sample and discuss its potential application to vegetation monitoring.

【P-57】

樹幹流に含まれる環境 DNA から樹上性ラン科植物の共生菌は検出できるか？

Can symbiotic fungi of epiphytic orchids be detected from environmental DNA in stemflow?

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ラン科植物は初期生育に共生菌を必要とするため、適切な共生菌が存在しない場所には定着できない。そのため、樹上性のランを効率的に保全するには、共生菌が存在する樹木の把握が重要である。しかし、樹上環境における共生菌の有無を、樹木単位で非破壊的に把握する実用的な手法は確立されていない。樹幹流は枝や幹の表面を流下して幹部へ到達する雨水であり、樹上に存在する共生菌の DNA を含む可能性がある。そこで本研究では、樹幹流環境 DNA による共生菌の検出可能性を検討した。東京大学千葉演習林で、着生ランが生育する樹木 4 本と生育しない樹木 1 本から、3 回の降雨で計 14 サンプルの樹幹流を採取し、重力濾過後のフィルターから DNA を抽出した。共生菌の特異的プライマーで PCR を行った結果、通常 PCR では明瞭な増幅が得られにくかった一方、Nested PCR により一部サンプルで目的サイズ付近の増幅産物が確認された。

Orchids depend on symbiotic fungi during their early developmental stages and therefore cannot establish in habitats where suitable fungal partners are absent. Efficient conservation of epiphytic orchids consequently requires the identification of trees that harbor their symbiotic fungi. However, no practical method has been established to determine the presence or absence of these fungi in arboreal environments at the individual-tree level without destructive sampling. Stemflow is rainwater that flows along the surfaces of branches and trunks and reaches the lower trunk, and it may contain DNA derived from symbiotic fungi inhabiting the tree canopy. In this study, we examined the feasibility of detecting orchid symbiotic fungi using environmental DNA from stemflow. At the University of Tokyo Chiba Forest, stemflow was collected from four trees supporting epiphytic orchids and one tree without epiphytic orchids during three rainfall events, yielding a total of 14 samples. DNA was extracted from filters after gravity filtration. PCR was performed using primers specific to orchid symbiotic fungi. Conventional PCR did not consistently produce clear amplification, whereas nested PCR yielded amplicons of approximately the expected size in some samples. In the presentation, we evaluate the effectiveness and limitations of this method for detecting orchid symbiotic fungi.

【P-58】

樹幹流の環境 DNA メタバーコーディング分析による森林昆虫群集の解析

Analysis of forest insect communities using eDNA metabarcoding of stemflow

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近年、樹幹流の環境 DNA 分析により、樹上に生息する地衣類や樹木周辺の哺乳類の環境 DNA を検出できることが明らかになっている。しかし、樹幹流を用いた昆虫群集評価の有効性については、これまで検証されていない。そこで本研究では、同一森林内の樹木から樹幹流を採取し、環境 DNA メタバーコーディングにより昆虫群集を解析した。現時点では、8 樹種 25 本の樹木から得られた樹幹流を解析し 9 目 57 種の昆虫の DNA が検出された。従来法の採集や同定が困難であった小型昆虫の DNA についても、樹幹流から検出されている。ここまでのデータ解析の結果からは、樹木間の β 多様性の大部分はターンオーバーによって説明され、樹幹流に含まれる環境 DNA が樹木ごとに異なる昆虫群集を反映している可能性が示唆された。発表当日までにデータを追加し、樹幹流を利用した非侵襲的な森林昆虫モニタリングの有効性について議論する。

Recent studies have demonstrated that DNA of arboreal lichens and mammals around trees can be detected by eDNA analysis of stemflow. However, the effectiveness of eDNA analysis of stemflow for assessing insect communities has not yet been evaluated. In this study, stemflow samples were collected from trees within a single forest, and insect communities were analyzed using eDNA metabarcoding. Based on the analyses completed to date, 57 insect species across 9 orders have been detected from stemflow samples which were collected from 25 individual trees across eight tree species. DNA of small insects that are difficult to collect and identify using conventional methods were also detected in stemflow samples. Current data analyses indicate that most of the β -diversity among trees is explained by species turnover, suggesting that eDNA contained in stemflow reflects tree-specific insect communities. Additional data will be incorporated before the presentation, and we will discuss the effectiveness of non-invasive monitoring of forest insect communities using eDNA analysis of stemflow.

【P-59】

降雨に依存しない樹上生物多様性モニタリング法の開発

Development of a rainfall-independent method for monitoring arboreal biodiversity

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高い生物多様性を有する樹上環境の生物相を簡便に把握するため、我々は樹幹流を用いたモニタリング法を開発してきたが、降雨時にしか試料を採取できない制約があった。そこで本研究では、降雨に依存しない新たなサンプリング法を開発した。樹幹に巻き付けたガーゼに精製水を噴霧し、樹幹上の生物片などを回収し、回収したガーゼを精製水に浸漬し、その浸漬液をろ過して抽出したDNAについて環境DNAメタバーコーディング解析を行った。地衣類をモデルとした検証の結果、降雨直後に実施した場合、本手法は従来法と概ね同等の検出結果を示した。一方で、降雨日から1から3日程度経過すると検出されなくなる種も認められた。降雨直後であれば従来法と概ね同等の検出結果が得られることから、本手法はより簡便な樹上生物多様性モニタリング法として活用できる可能性が示された。

To facilitate simple monitoring of arboreal biodiversity, one of the most species-rich components of terrestrial ecosystems, we previously developed a monitoring method using stemflow. However, this method is limited by the fact that samples can only be collected during rainfall events. To overcome this limitation, we developed a rainfall-independent sampling method. Purified water was sprayed onto gauze wrapped around tree trunks to collect biological material from the trunk surface. The recovered gauze was then soaked in purified water, and the resulting solution was filtered for DNA extraction and subsequent environmental DNA (eDNA) metabarcoding. Validation using lichens as a model group showed that, when applied shortly after rainfall, the new method generally yielded detection results comparable to those obtained using the conventional stemflow method. However, some species were no longer detected one to three days after rainfall. Nevertheless, because the new method yielded detection results broadly comparable to those obtained using the conventional method when applied shortly after rainfall, it has the potential to serve as a simpler method for monitoring arboreal biodiversity.

【P-60】

樹液および人工トラップ由来環境 DNA による好樹液性昆虫と微小生物相の検出

Detection of sap-associated insects and micro-biodiversity using environmental DNA from tree sap and artificial traps

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陸域における環境 DNA の応用は発展途上であり、森林昆虫相を簡便かつ低侵襲に把握する手法の確立が求められる。本研究では、樹液に集まる生物の DNA が樹液やこれを模した人工トラップ表面に残留・蓄積するという仮説を検証した。大学の学校林などの森林でスワブ採取を行い、COI 領域および gInsect プライマー（16S rRNA 領域）を用いた DNA メタバーコーディングを実施した。その結果、複数の好樹液性昆虫を検出し、既存調査で確認された種に加え、地域で記録の少ない種も確認された。さらに線虫、ダニ類、昆虫病原菌なども検出され、樹液が昆虫のみならず微小生物や寄生・共生関係を含む生物相の DNA を蓄積する場である可能性が示された。将来的には、本手法を既存の目視調査やカメラ調査と組み合わせることで、森林・緑地の生物多様性を簡便かつ継続的に評価するための標準的なモニタリング手法へ発展させたい。

Applications of environmental DNA (eDNA) in terrestrial ecosystems are still developing, and there is a need to establish simple and minimally invasive methods for monitoring forest insect assemblages. In this study, we tested the hypothesis that DNA from organisms visiting tree sap remains and accumulates in natural tree sap and on the surfaces of artificial traps that mimic tree sap. Swab samples were collected from forests, including university-owned school forests, and DNA metabarcoding was conducted using primers targeting the COI region and the gInsect primer targeting the 16S rRNA region. As a result, multiple sap-associated insect species were detected, including species previously confirmed by conventional surveys as well as species rarely recorded in the region. In addition, nematodes, mites, and entomopathogenic fungi were also detected, suggesting that tree sap may serve as a substrate that accumulates DNA not only from insects but also from micro-organisms and biological communities involving parasitic and symbiotic relationships. In the future, we aim to develop this method into a standardized monitoring approach for simply and continuously assessing biodiversity in forests and green spaces by combining it with conventional visual surveys and camera-based surveys.

【P-61】

環境 DNA 分析を用いた訪花昆虫相解明のためのサンプリング法の確立

Establishment of eDNA sampling method for revealing flower-visiting insect communities

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花は多様な昆虫に、餌資源や産卵場所、微小生息環境を提供している。訪花昆虫相を明らかにすることは、花の生態系機能の解明や植物 - 訪花昆虫群集の保全のために重要である。従来、訪花昆虫の種同定には高度な分類学的知識が求められていた。花上の環境 DNA を回収し、解析することで、簡便に訪花昆虫相を明らかにできる可能性があるが、サンプリング法の侵襲性や検出感度に課題がある。本研究では、環境 DNA 分析を用いた訪花昆虫相把握に向けた基盤を構築するために、最適なサンプリング方法を検討した。花上の環境 DNA の採取法として、侵襲的な花全体の切除と、非侵襲的な水による表面洗浄と綿棒による表面払拭、の3手法を比較した。また、各採取法で1サンプルで扱う花の数と検出感度の関係を調べた。本発表では、採取法と1サンプル内の花数の違いが検出種数や組成に及ぼす影響を検討し、訪花昆虫相の解明に適したサンプリング方法について議論する。

Flowers provide food resources, oviposition sites, and microhabitats for a wide variety of insects. Identifying flower-visiting insects is important for maintaining ecosystem functions and conservation of biodiversity. The conventional way to identify them requires detailed morphological observation and a high level of taxonomic expertise. Insect communities can be revealed easily by collecting and analyzing insect-derived eDNA remaining on flowers. However, current sampling methods have limitations in terms of invasiveness and detection sensitivity. In this research, we investigated the optimal method for collecting eDNA on flowers to establish the basis for understanding communities of flower-visiting insects using eDNA analysis. We compared three sampling methods. One is an invasive method, collecting whole flowers, and the others are non-invasive methods; washing off the flower surface with water and swabbing the flower surface. We also examined how the number of flowers included in a single sample affects detection sensitivity. In this presentation, we evaluate whether each eDNA sampling methods and the number of flowers included in a single sample affect the number of detected taxa and the composition of functional groups. Based on these results, we discuss the optimal sampling strategy for characterizing flower-visiting insect communities using eDNA analysis.

Development of a Sampling Methods for Arthropod eDNA in Grasslands

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Environmental DNA (eDNA) metabarcoding has emerged as a powerful tool for monitoring arthropod communities without capturing and morphologically identifying individual specimens. However, existing sampling methods are not well suited for efficiently collecting arthropod eDNA across large areas of heterogeneous grassland vegetation. To address this limitation, we developed a roller-based method for collecting arthropod eDNA from grasslands. In this method, a rigid polyvinyl chloride (PVC) tube wrapped in gauze is attached to the tip of a sticky lint roller; rolling this device over the grassland vegetation allows eDNA to be adsorbed onto the gauze. The gauze is then rinsed with distilled water, and the resulting solution is filtered through a Sterivex cartridge to collect eDNA for subsequent DNA extraction. We evaluated the performance of this roller-based sampling method by comparing it with the existing sampling method Plant flow collection (PFC) method. PCR amplification was successful for samples collected using the PFC method but was inconsistent for samples collected using the roller-based method. Ongoing efforts to optimize PCR amplification will also be presented.

【P-63】

河川環境 DNA による森林集水域優占樹種の遺伝的多様性評価

Assessing the genetic diversity of dominant tree species in forest watersheds using stream environmental DNA

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河川水中の環境 DNA には、集水域に生育・生息する生物の遺伝的多様性を反映した情報が含まれていると考えられる。本研究では、環境 DNA に MIG-seq 法を適用し、森林河川の環境 DNA によって集水域における優占樹種の遺伝的多様性を評価した。調査地として、冷温帯落葉広葉樹林を流れる 2 河川の源流から下流にかけて計 7 地点を設定し、河川水の環境 DNA サンプルングを冬期以外 2 年間にわたり毎月 1 回実施した。解析対象種には、調査地林内の主要構成樹種であるブナを選定した。得られた環境 DNA 由来のリードをブナの MIG-seq リファレンス配列にマッピングし、塩基多様度を算出した。その結果、塩基多様度は上流で低い傾向を示し、落葉期には高い値になる傾向が認められた。本手法は、環境 DNA による遺伝的多様性評価の新たなアプローチとして、森林生態系の遺伝的モニタリングや様々な生物群への応用が期待される。

Environmental DNA (eDNA) in streams has the potential to reflect the genetic diversity of organisms inhabiting a watershed. In this study, we applied the genome-wide SNP genotyping method MIG-seq to stream eDNA to assess the genetic diversity of a dominant tree species in a forest watershed. eDNA samples were collected monthly (excluding winter) for two years from seven sites distributed from the headwaters downstream in two forest streams. Japanese beech (*Fagus crenata*), the dominant tree species in the study area, was selected as the target species. Sequence reads obtained from eDNA were aligned to a *F. crenata* reference dataset, and nucleotide diversity was estimated. Nucleotide diversity tended to be lower at upstream sites and higher during the leaf-fall season. These results demonstrate the potential of stream eDNA for assessing the genetic diversity of dominant tree species and provide a new approach for eDNA-based genetic monitoring of forest ecosystems.

【P-64】

環境 DNA 由来の樹種候補情報を活用した広葉樹林リモートセンシング MRV 手法の検討

—静岡市 Verra VM0047 プロジェクトにおける予備検証—

Integrating Environmental DNA-Derived Tree Species Information into Remote-Sensing MRV of Broadleaf Forests: A Preliminary Study within a Verra VM0047 Project in Shizuoka City

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リモートセンシングを用いた森林 MRV は、比較的樹種構成が単純な針葉樹林を中心に発展してきた一方、広葉樹林では樹種分類に用いる信頼性の高い教師データの確保が課題である。本研究では、静岡市で進める Verra VM0047 プロジェクトを対象に、環境 DNA (eDNA) から得られる候補樹種情報を、ドローン LiDAR、マルチスペクトル画像、地上・バックパック LiDAR と統合し、広葉樹林における carbon MRV の高度化を検討する。予備調査では植物 40 種、哺乳類 3 種、淡水魚 2 種、昆虫 10 種を検出した。今後、eDNA 検出結果、採取地点の集水域、現地植生調査、リモートセンシングデータの対応関係を検証し、森林区分、炭素蓄積量推定、モニタリング精度の向上への有効性と限界を評価する。さらに、本手法を生物多様性 MRV や自然関連評価へ展開する可能性を検討する。

Remote-sensing-based forest MRV has advanced mainly in relatively homogeneous coniferous stands, while its application to species-rich broadleaf forests remains challenging because reliable training data for tree-species classification are difficult to obtain. This study proposes a novel approach using environmental DNA (eDNA) as a complementary source of training data for remote-sensing-based carbon MRV. Within a Verra VM0047 project in Shizuoka City, Japan, we integrate eDNA-derived candidate tree-species information with drone LiDAR, multispectral imagery, and terrestrial and backpack LiDAR data. Preliminary eDNA surveys detected 40 plant species, 3 mammal species, 2 freshwater fish species, and 10 insect species. The next phase will assess relationships among eDNA detections, sampling-site catchments, field vegetation observations, and remote-sensing data. By improving information on tree-species composition and forest structure, this approach may strengthen forest stratification, carbon-stock estimation, and monitoring reliability in complex broadleaf forests, thereby supporting operational carbon MRV for VM0047 projects. The framework may also provide a basis for integrating carbon and biodiversity MRV within broader nature-related assessment frameworks.

【P-65】

土壤中植物 DNA を用いた法科学的土壌識別

Forensic discrimination of soil samples by plant DNA metabarcoding

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土壌は身の回りに存在し、犯罪を解明するための鑑定資料となることがある。例えば被疑者に付着していた土壌が犯罪現場に由来するか否かを判断できれば、犯罪への関与の判断に役立つ。本研究では土壌中の植物 DNA に着目し、環境 DNA による土壌の法科学的な識別法の開発を試みた。

関東地方の異なる作物が栽培されていた家庭菜園から土壌を 7 点採取した。DNA 抽出後、葉緑体のコピー数を定量し、7 点の試料の鋳型量をそろえた上で、葉緑体上の 2 領域 (*trnL*, *rbcL*) についてメタバーコーディング解析を実施した。解析は 3 回行った。

メタバーコーディングで得られた配列は殆どがその場所で栽培されていた作物であった。また、3 回の解析結果から類似度指数を計算した結果、栽培されていた作物毎に識別できることが示唆された。従来識別が困難であった土壌の識別が可能となる可能性があり、法科学分野における土壌鑑定への有用性が期待される。

Since soil is present in our immediate surroundings, it can sometimes serve as forensic evidence. If soil found on a suspect is proven to originate from a crime scene, it can be useful evidence to associate the suspect with the scene. In this study, we attempted to develop a forensic method for soil identification using plant DNA metabarcoding.

We collected seven soil samples from home gardens in the Kanto region where different crops were being cultivated. After DNA extraction, we quantified the chloroplast copy number and standardized the template amount across the seven samples before performing metabarcoding analysis on two chloroplast regions (*trnL* and *rbcL*). The analysis was performed three times.

The sequences obtained through metabarcoding were almost exclusively from the crops cultivated at those locations. Furthermore, the calculation of similarity indices based on the results of the three analyses suggested that each cultivated crop could be discriminated. This approach may enable the discrimination of soil samples that were previously difficult to discriminate and is expected to be useful for soil analysis in the field of forensic science.

Integration of Data and Abiotic Data for Predicting the Distribution of Indo-Pacific Finless Porpoises in Hong Kong Waters

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The Indo-Pacific finless porpoise (*Neophocaena phocaenoides*) is a coastal cetacean whose habitat use is likely shaped by prey availability, yet conventional survey methods cannot simultaneously resolve predator occurrence and prey community dynamics. This study applied a territory-wide environmental DNA (eDNA)-based approach across Hong Kong waters to investigate how prey fish communities and abiotic factors influence the spatial distribution of the finless porpoise. Porpoise eDNA was detected using species-specific qPCR and eDNA metabarcoding, while prey fish communities were characterized by quantitative eDNA metabarcoding with internal DNA spike-in to estimate the absolute eDNA copy numbers. Simultaneously, *in-situ* environmental parameters (sea surface temperature, salinity, and dissolved oxygen) were also measured during sampling. By integrating these biotic (fish community eDNA) and abiotic (environmental parameters) datasets, we estimated their contributions to predictions of the finless porpoise distribution. We found that spatial variations in prey fish communities, particularly carnivorous fish and medium-sized fish, were associated with the detectability of the finless porpoise eDNA. Besides, we calculated cumulative fish eDNA copy numbers around the sampling locations and found that models including the environmental parameters and the “cumulative” fish eDNA indices explained the finless porpoise eDNA detection better than models with the environmental parameters only. These results suggested that prey fish eDNA data, particularly spatially cumulative indices, can improve the prediction of the detectability of the finless porpoise eDNA in Hong Kong waters.

Climate-Driven Northward Retreat Meets Vertical Constraints: eDNA Profiling Reveals 3D Habitat Compression for Polar Cod

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Global warming is restructuring Arctic marine ecosystems at an unprecedented pace. As a central trophic hub, linking pelagic-benthic food webs and lower-to-higher trophic levels, Polar cod (*Boreogadus saida*) is critical to maintaining the stability and function of Arctic ecosystems. Understanding how its distribution responds to climate change is essential for forecasting ecosystem shifts and informing conservation strategies. Here, we investigated the vertical and horizontal distribution patterns of Polar cod using environmental DNA (eDNA) profiles collected during autumn cruise surveys (2020–2023) across the Bering, Chukchi, and Beaufort Seas. Vertical profiling revealed a distinct depth-stratified pattern: eDNA occupancy rates dropped in the middle and bottom layers of the Arctic Basin (54.2% and 60%), contrasting with consistently high detection throughout shelf and slope waters and the Basin's surface (80%). Horizontal ensemble species distribution modeling (ESDM) showed that habitat suitability is currently low in the Bering Sea but remains high in the Chukchi and Beaufort Seas, driven primarily by sea ice dynamics, salinity, and temperature. Under future climate projections, the southern habitat boundary contracts northward, confining Polar cod to the Arctic Basin. Crucially, the discovery of this pelagic “middle desert” in the Arctic basin inferred from low eDNA occupancy rates suggests that as climate change forces Polar cod northward into the deep Basin, they might face severe vertical habitat compression. These findings highlight the power of eDNA profiling in uncovering hidden ecological constraints and offer new insights into the climate vulnerability of Arctic marine life.

【P-68】

環境 DNA メタバーコーディングによって明らかになった瀬戸内海のクモヒトデ類の季節性

Seasonal variation in brittle star detection in the Seto Inland Sea, Japan, revealed by environmental DNA metabarcoding

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環境 DNA (eDNA) メタバーコーディングは海洋生物多様性の非侵襲的モニタリング手法だが、底生無脊椎動物への適用例は乏しい。本研究では棘皮動物門クモヒトデ綱特異的プライマー 16SOph1 を用い、瀬戸内海の広島県竹原（中央部）と徳島県鳴門（東部入口）の 2 地点で四季にわたり eDNA 解析を行った。表層水を濾過し、ミトコンドリア 16S rRNA 遺伝子を解析した結果、四季を通じて竹原では 12 種、鳴門では 9 種が検出された。検出種数は両地点ともに季節によって異なり、夏に最大（竹原 11 種、鳴門 8 種）で、他季節は 1～4 種の検出にとどまった。これらのことから、クモヒトデ類の eDNA 調査においては、夏季に採水することが最も効率的である可能性が示唆され、底生無脊椎動物の eDNA モニタリングにおいて季節性を考慮する重要性が示された。

Environmental DNA (eDNA) metabarcoding is a powerful non-invasive tool for monitoring marine biodiversity, yet its application to benthic invertebrates remains limited. Brittle stars (Ophiuroidea: Echinodermata) are species-rich, ecologically dominant, and well-suited to eDNA detection. We conducted eDNA metabarcoding at two sites in the Seto Inland Sea, Japan—Takehara, Hiroshima (central) and Naruto, Tokushima (eastern entrance) —across four seasons (spring, summer, autumn, and winter). Surface-water samples were filtered for analysis of the mitochondrial 16S rRNA gene. Across the four seasons, 12 brittle star species were detected at Takehara and nine at Naruto. The number of detected species varied among seasons at both sites and was highest in summer, with 11 species detected at Takehara and eight at Naruto, whereas only one to four species were detected in the other seasons. These findings suggest that summer may be the most efficient single sampling season for ophiuroid eDNA surveys and highlight the importance of considering seasonality when designing eDNA monitoring surveys for benthic invertebrates.

Biotic and abiotic controls on historical population dynamics of the copepod *Paracalanus orientalis* revealed by sedimentary DNA

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Environmental DNA in sediment cores (sedDNA) has been increasingly used as a powerful tool for reconstructing historical zooplankton population dynamics in lakes and freshwaters; however, its applicability to marine copepods remains largely unexplored. In the present study, we evaluated the potential of marine sedDNA to reconstruct the long-term population dynamics of the copepod *Paracalanus orientalis* and examined the influences of environmental variables (temperature, salinity, dissolved oxygen, nutrients, and chlorophyll *a* concentrations) and predator pressure from pelagic fishes. Two sediment cores were collected from the western and eastern parts of Harima-Nada in November 2025, and species-specific quantitative PCR was used to quantify sedDNA concentrations of *P. orientalis*. Based on the preliminary age model, *P. orientalis* sedDNA concentrations remained high from the late 1980s to the 2000s in the western part of Harima-Nada and were significantly correlated with salinity and dissolved oxygen. They were also negatively correlated with the stock dynamics of Japanese anchovy (Seto Inland Sea stock) and Japanese sardine (Pacific stock). In eastern Harima-Nada, sedDNA concentrations remained relatively low from the 1980s to the middle 2010s but increased thereafter. Here, sedDNA concentrations were negatively correlated with chlorophyll *a* concentration and sand lance catches, while showing a positive relationship with Japanese sardine stock. These findings demonstrate the applicability of sedimentary DNA for reconstructing long-term dynamics of marine copepods and provide new insights into the combined influence of environmental variability and predator–prey interactions on marine zooplankton populations.

【P-70】

Nanopore 長鎖メタゲノムから抽出した完全長 18S rDNA による海洋尾索動物の識別

Identification of marine tunicates using full-length 18S rDNA sequences extracted from Nanopore long-read metagenomic data

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18S rDNA 短鎖領域を用いた環境 DNA メタバーコーディングでは、広範な真核生物を検出できる一方、近縁種の識別が難しい場合がある。本研究では、沿岸海洋生態系の長期観測基盤 PlanDyO で取得した Nanopore 長鎖メタゲノムリードから、PCR を介さずに完全長 18S rDNA に相当する配列を抽出し、海洋尾索動物の識別可能性を検討した。81 試料、約 1.13 億リードから 3,097 配列を得て、MAFFT による整列と K-medoids クラスタリングを行った結果、*Oikopleura* 属 4 既知種に近縁な群を含む尾索動物 10 系統と、既存参照配列に明確に対応しない群が得られた。また、*O. dioica* に帰属された配列は複数群に分かれ、その群間差は推定シーケンシングエラーを上回った。完全長配列では V9 領域のみでは捉えにくい群間差が認められ、短鎖メタバーコーディングを補完しうることが示唆された。

Environmental DNA metabarcoding using short 18S rDNA markers can detect a broad range of eukaryotes, but may not resolve closely related species. In this study, we extracted full-length 18S rDNA sequences without PCR amplification from Nanopore metagenomic long reads generated by PlanDyO, a long-term coastal marine ecosystem monitoring platform, and evaluated their utility for identifying marine tunicates. From approximately 113 million reads from 81 samples, 3,097 full-length sequences were obtained and analyzed using MAFFT alignment and K-medoids clustering. The analysis recovered 10 putative tunicate lineages, including groups closely related to four known *Oikopleura* species and groups without clear matches to existing reference sequences. Sequences assigned to *O. dioica* were separated into multiple groups, and the distances among these groups exceeded the estimated contribution of sequencing errors. Full-length sequences also resolved differences among groups that were difficult to distinguish using the V9 region alone. These results suggest that full-length 18S rDNA sequences extracted from Nanopore metagenomic data can complement short-amplicon metabarcoding.

【P-71】

環境 DNA メタバーコーディングで読み解く舞鶴湾内における魚類群集の三次元構造

Revealing the Three-Dimensional Structure of Fish Communities in Maizuru Bay Using Environmental DNA Metabarcoding

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水温や塩分などによって形成される水塊構造は、魚類群集の空間構造を規定する重要な要因と考えられている。しかし、魚類群集の水平・鉛直構造を高い空間解像度で評価した研究は少ない。本研究では、日本海に面する舞鶴湾において、100 定点の 3 水深層（表層・中層・底層）から計 300 試料の環境 DNA を採取し、環境 DNA メタバーコーディングにより魚類群集を解析した。その結果、216 魚種が検出され、魚類群集組成は 3 水深層間で顕著に異なるとともに、湾内の水平方向にも空間的な差異が認められた。これらの結果から、半閉鎖性内湾において魚類群集が明瞭な三次元的空間構造を形成していることが示された。以上より、環境 DNA メタバーコーディングは、沿岸生態系の魚類群集を高い空間解像度で評価するための有効な手法であることが示された。

Hydrographic structure, characterized by environmental gradients such as water temperature and salinity, is considered an important factor shaping the spatial organization of fish communities in coastal bays. However, few studies have evaluated the horizontal and vertical structure of fish communities at high spatial resolution. In this study, we applied quantitative environmental DNA (eDNA) metabarcoding to investigate fish community structure in Maizuru Bay, a semi-enclosed coastal bay facing the Sea of Japan. eDNA samples were collected from three depth layers—surface, middle, and bottom—at 100 stations, yielding a total of 300 samples. Fish community data were compared with environmental variables measured simultaneously using a multiparameter water-quality profiler. A total of 216 fishes were detected. Fish community composition differed markedly among the three depth layers and exhibited gradual spatial turnover along the horizontal axis of the bay. These results demonstrate that fish communities exhibit clear three-dimensional spatial structuring within a semi-enclosed coastal bay. Overall, our findings demonstrate that eDNA metabarcoding is an effective approach for assessing coastal fish community structure at high spatial resolution.

Species association structure enhances compositional resistance to short-term warming in marine fish communities

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Anthropocene climate warming is reshaping the composition of ecological communities and altering patterns of species co-occurrence. While extensive research has shown how climatic extremes reshape species associations, whether pre-existing association structure renders communities more susceptible or more resistant to warming-induced destabilization remains underexplored. Here, we leveraged environmental DNA time-series data from nine coastal sites across Japan, spanning 24.42 N to 43.04 N from 2017 to 2021, to test whether within-community species association structure, quantified as cohesion, modulates compositional reorganization under short-term warming of up to 120 days. We found that communities with stronger negative cohesion were more resistant to warming-induced compositional destabilization. This modulating effect, however, diverged between thermal subcommunities: stronger negative cohesion enhanced compositional resistance in warm-affinity fishes but amplified compositional change in cold-affinity fishes. These findings suggest that species association structure can provide insurance effects against warming-induced reorganization, while simultaneously amplifying the compositional divergence between warm- and cold-affinity taxa. Our study underscores the need to account for both species association structure and the thermal affinities of species when anticipating marine biodiversity responses to warming.

Assessing the Effects of Urbanization on Coastal Fish Communities Using Large-Scale Data

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沿岸域は陸域からの栄養塩供給によって高い生産性を有するものの、人間活動の影響を受けやすい。近年の急速な都市開発による沿岸域生物多様性への影響が懸念されている。地中海において都市度が高い地域ほど魚類群集が均質化し、多様性が損失していることが報告されている。日本は世界でも有数な漁場であり、沿岸魚類は生態系サービスを支える重要な存在である。しかし、都市度が沿岸魚類群集に及ぼす影響は十分に解明されていない。本研究では、環境 DNA メタバーコーディングによる日本沿岸の大規模魚類群集データを用い、都市度と魚類群集との関連を調べた。地点間の群集組成の類似度を調べたところ、都市では魚類群集が比較的類似していた。また、水分や塩分などの環境への群集応答が、都市度の高いグループでは、低都市度のグループより有意に鈍化しており、都市における群集の均質化が示唆された。これらの結果は、都市の生物多様性保全の一助となる。

Coastal areas exhibit high productivity due to nutrient input from land, yet they are highly susceptible to human activity. The impacts of rapid recent urban development on coastal biodiversity are a growing concern. In the Mediterranean Sea, fish communities have been reported to become more homogeneous and to lose diversity in areas with higher urbanization. Japan is known as one of the world's leading fishing grounds, and coastal fish play an important role in supporting ecosystem services. However, how urbanization affects coastal fish communities remains insufficiently understood. Here, we used large-scale fish community data obtained through environmental DNA metabarcoding across Japan's coastal waters to examine the relationship between urbanization and fish communities. Comparing community composition similarity among sites, we found that fish communities were relatively similar among Japan's three major metropolitan areas; Tokyo, Nagoya, and Osaka. We also found that the response of community composition to environmental variables such as salinity and water temperature was significantly weaker in the high-urbanization group than in the low-urbanization group, suggesting biotic homogenization in urban areas. These findings may contribute to efforts to conserve biodiversity in urban environments.

【P-74】

環境 DNA 大規模観測による海洋環境変動に対する三陸沿岸魚類群集の時空間的応答

Spatiotemporal responses of coastal fish communities to ocean environmental variability revealed by large-scale eDNA monitoring

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近年の大規模な海流変動は、沿岸環境や生態系を変化させる要因となる。三陸沿岸では 2023 年以降、黒潮続流の北方流入に伴う広域的な水温上昇が生じているが、これに対する魚類群集の応答が沿岸域の位置によってどのように異なるかは明らかでない。本研究では、2022 年秋から年 3 回、三陸沿岸（宮古～女川）の 18 湾 54 地点で環境 DNA メタバーコーディングによる魚類群集調査を実施した。PCoA により群集組成を要約し、各地点の PCoA 軸スコアの経年変化を比較した。その結果、主要 2 軸のスコアは水温変動に伴って変化し、環境変動に伴う群集組成の変化を捉えていることが示唆された。また、2025 年には水温が平年並みに戻ったものの、群集組成の変化は一様ではなく、軸や調査地点の緯度によって、水温上昇前の状態への回復や、水温上昇期の状態の維持など異なるパターンを示した。

Large-scale changes in ocean currents can substantially alter coastal environments and ecosystems. Along the Sanriku coast, sea surface temperatures have increased extensively and persistently since 2023, following the northward intrusion of the Kuroshio Extension. However, it remains unclear whether and how fish communities respond differently to such environmental changes across geographically distinct coastal areas. We conducted eDNA metabarcoding surveys of fish communities from autumn 2022 onward at 54 sites across 18 bays along the Sanriku coast, from Miyako to Onagawa, with three surveys conducted annually thereafter. We used PCoA to summarize community composition and examined interannual changes in PCoA axis scores across sites. The two major PCoA axis scores fluctuated in association with changes in water temperature, suggesting that they reflected temporal changes in community composition associated with environmental variability. Although sea surface temperatures returned to near-average levels in 2025, community composition did not show a consistent pattern of recovery. Depending on the PCoA axis and latitude, some communities shifted toward conditions observed before the warming period, whereas others remained similar to those observed during the warming period. The different responses observed among PCoA axes and latitudes suggest that fish community responses to warming are not only spatially heterogeneous but may also differ among species. Future analyses will identify the species that contribute most strongly to each PCoA axis and examine their ecological characteristics to explore the potential drivers of these differences in community responses.

Latitudinal and Thermal Drivers of Deep-Sea eDNA Phenology in Coastal Japan

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Research into deep-sea fish has been highly limited because of financial and infrastructural constraints. Hence, providing us very little information about these organisms' movement or preferred habitable conditions. Can coastal water samples unlock the biodiversity of the deep ocean? While deep-sea environmental DNA (eDNA) is occasionally detected in coastal bays, the physical and oceanographic drivers of this phenomenon are heavily debated. Utilizing the AnemoneDB, we used a 5-year eDNA time-series across the Japanese archipelago and tracked 44 deep-sea taxa to test whether coastal detection is driven by physical geomorphology (steep submarine slopes), temporal changes leading to vertical migration or oceanographic mixing.

By integrating NOAA bathymetry and NASA satellite telemetry into generalized linear mixed models (GLMMs) and community ecology networks, we reveal that submarine slope steepness has no significant effect on deep-sea eDNA abundance. Instead, evidence points towards temporal changes in regional water masses and the seasonal breakdown of thermal stratification acting as absolute gatekeepers. Dividing the Japanese archipelago into 3 regions North, Center and South, richness based PERMANOVA was conducted which proved statistically significant. Further analysis might reveal evidence of the thermal niche of deep-sea fish.

【P-76】

海洋微生物群集のメタゲノム配列特徴量を用いた沿岸環境変数の推定

Estimation of coastal environmental variables from metagenomic sequence features of marine microbial communities

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海洋微生物群集は、水温や栄養塩などの環境条件に応じて変動するため、メタゲノム配列には海洋環境と関連する情報が含まれる可能性がある。そこで本研究では、メタゲノム配列からどのような海洋環境変数を推定できるかを検討した。解析には、PlanDyO プロジェクトにおいて女川湾、石巻湾、陸奥湾の計9観測ステーションから月次採取した76試料のメタゲノム配列とCTD観測データを用いた。分類群の同定を介さず、配列から得たk-mer頻度などを特徴量として、機械学習により6種類の環境変数を推定した。5分割交差検証の結果、水温、溶存酸素、クロロフィルa、密度は一定程度推定できた一方、塩分と濁度の推定は困難であった。また、予測に寄与する6-merは環境変数ごとに異なった。これらの結果は、海洋微生物群集の配列特徴と環境条件との対応関係が、環境変数ごとに異なることを示唆する。

Marine microbial communities vary in response to environmental conditions such as water temperature and nutrient concentrations, suggesting that metagenomic sequences may contain information associated with the marine environment. We investigated which marine environmental variables could be estimated from metagenomic sequence features. We analyzed metagenomic sequences and CTD observations from 76 samples collected monthly at nine stations in Onagawa Bay, Ishinomaki Bay, and Mutsu Bay through the PlanDyO project. Without taxonomic assignment, k-mer frequencies and pretrained sequence embeddings were used as input features for machine learning models to estimate six environmental variables. Five-fold cross-validation indicated that temperature, dissolved oxygen, chlorophyll a, and water density could be estimated to some extent, whereas salinity and turbidity were difficult to estimate. In addition, the 6-mers contributing to prediction differed among environmental variables. These results suggest that the associations between metagenomic sequence features of marine microbial communities and environmental conditions differ among environmental variables.

Multitaxa eDNA Metabarcoding for Mapping Hidden Biodiversity and Potential Community Interactions in Ramsar Mangrove Wetland

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Global mangrove loss has made effective conservation and restoration urgent—especially within protected areas such as Ramsar sites. To support sustainable management, it is essential to understand spatial patterns of biodiversity and their trophic dynamics within protected mangrove reserves. Here, we investigate “hidden” biodiversity using eDNA metagenomics in the Mai Po Nature Reserve, one of Hong Kong’s largest mangrove ecosystems and an early Ramsar-listed site (~400 ha), protected for over 40 years. The reserve sustains 60,000 migrating waterbirds annually, reflecting its high productivity and biodiversity. Our multitaxa eDNA survey detected over 2,000 taxa spanning multiple trophic levels. At fine spatial and temporal resolution, we also distinguished mudflat skippers to characterize seasonal distribution patterns. Because traditional community-interaction approaches may miss low-abundance taxa, we applied multiple marker genes to reconstruct putative, cross-trophic interaction networks and identify keystone taxa. Overall, our results demonstrate that eDNA metabarcoding can reveal cryptic mangrove biodiversity and candidate species interactions, offering actionable insights into ecosystem structure, stability, and conservation planning.

Fish biodiversity across tidal conditions in the Urauchi River subtropical mangrove estuary revealed by eDNA metabarcoding

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Mangrove estuaries present significant challenges for fish biodiversity assessment owing to strong tidal fluctuations and complex habitat structure. Environmental DNA (eDNA) metabarcoding offers a promising approach for assessing fish communities in these dynamic ecosystems. We investigated whether tidal conditions influence eDNA-derived diversity metrics and fish assemblage composition in the Urauchi River, Iriomote Island, Japan, using a waterline-tracking sampling design that repositioned sampling at the tidal waterline across four tidal phases during spring and neap tidal cycles. Four sampling campaigns conducted at two sites in April 2021 and September–October 2023 generated 32 eDNA samples, which were amplified using MiFish universal primers. Approximately 24 million quality-filtered reads yielded 337 operational taxonomic units (OTUs) representing 169 genera and 76 families, including 20 taxa listed on the IUCN, Japanese, and Okinawa Red Lists. OTU richness was significantly higher during spring tides. Multivariate analyses revealed structured variation in fish assemblage composition; NMDS ordination (stress = 0.13) visualised assemblage clustering among sampling periods, dbRDA explained 52.6% of total compositional variation (adjusted $R^2 = 0.301$, $p = 0.001$) with year and water temperature as the strongest drivers. PERMANOVA confirmed significant effects of location, tidal conditions, salinity, and distance from the river midpoint (all $p \leq 0.036$). These findings provide a molecular baseline of fish diversity for the Urauchi River mangrove estuary and demonstrate that spring-tide sampling enhances species detection, supporting tidal-aware eDNA long-term monitoring in conservation-priority mangrove ecosystems where robust species inventories are required for evidence-based management decisions.

【P-79】

外洋性魚類および深海性サンゴ類の環境 DNA 採集方法に関する予備的研究

Preliminary analysis of eDNA sampling methods targeting on high sea fish and coral assemblages

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環境 DNA（以後 eDNA）分析の手法は近年急速に技術開発が進んでおり、その適用は浅海域や陸域のみにとどまらず、外洋域における深海生態系の解明にも大きく寄与できることが期待されている。しかし、外洋域や深海域での適用例は未だ少なく、これらの海域における有効な研究手法も確立していない。そのため、本発表では、複数個所で採取した外洋性魚類および深海性サンゴ類 eDNA サンプルを対象とし、採水時の水深や海底からの距離の影響に着目した GLMM 解析を行った。その結果、リード数、および OTUs (Operational Taxonomic Units) と採水位置との関係性は、対象生物の生態（表層性 / 中深層性 / ベントス）の違いによって傾向が異なっており、特に外洋域での調査においては、対象とする生物の生態に着目して採水水深を検討する必要があることが示唆された。

Environmental DNA (eDNA) analysis has been rapidly advancing in recent years, and its value is not only for ecological studies in shallow waters or terrestrial environments but also contributes to our understanding of deep-sea ecosystems in the open ocean. However, there are currently few examples of its application in the open ocean or deep-sea, and our knowledge of effective research methods are still scarce in these fields. Therefore, in this presentation, we conducted a GLMM analysis to reveal the differences in abundance/species diversity of eDNA (fishes and octocorals) collected in the open ocean by focusing on the sampling positions (especially “depth” and “height from the seafloor”). Our analyses revealed that the trends of identified total number of reads and OTUs (Operational Taxonomic Units) varied among target organisms with different lifestyles (epipelagic/mesopelagic/benthos). Overall, our study suggested the importance of considering the lifestyles of target assemblages when deciding an effective sampling depth in the open ocean.

【P-80】

与論島における浅いサンゴ礁と深いサンゴ礁における環境 DNA メタバーコーディング解析からイシサンゴ群集の水深に応じた多様性の低下と変化が明らかになった。

eDNA metabarcoding in shallow and mesophotic reefs reveals declines in diversity and changes of compositions in scleractinian coral assemblages with depth in Yoron Island

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サンゴ礁に生息するサンゴの種類は、そこに生息する多くの生物の種類に影響する生態学的に重要な情報である。しかし、形態による同定の難しさから、分類学的に詳細な情報を含むサンゴのモニタリング調査が行われることは稀である。我々は、日本に生息するとされる約 85 属の中の 83 属のイシサンゴを検出できる eDNA メタバーコーディング法を開発し、各地でサンゴ礁調査を行っている。我々は、沖縄島の北にある、与論島で水深の浅い珊瑚礁と深いサンゴ礁で eDNA によるサンゴ礁調査を行った。水深の深いサンゴ礁は調査が困難であり、知見が不足している。調査結果から、サンゴ礁におけるイシサンゴの検出属数は水深に応じて減少することが示された。多くのイシサンゴの属は、浅いサンゴ礁で、多く検出されたが、熱ストレスに弱いトゲサンゴは深いサンゴ礁で多く見つかった。このことは、深いサンゴ礁が高温に対する refugia である説に合致する。

Scleractinian corals are key animals for tropical and subtropical seas because they sustain biodiversity of coral reefs. Reef monitoring projects are conducted all over the world for accessing coral reef health, however those with detail coral taxonomic information are rare because of difficulty of taxonomical identification of corals by morphology. We have developed an eDNA metabarcoding method capable of detecting 83 of the 85 scleractinian coral genera known to occur in Japan, and we are conducting reef surveys across various locations. Here we report results of scleractinian eDNA metabarcoding study on both shallow and deep coral reefs in Yoron Island, located north of Okinawa Island. Knowledge regarding mesophotic reefs (30-100 m in depth) remains limited because of difficulty of visual surveys by diving. Our results showed that the number of scleractinian genera detected decreased with increasing depth. While many scleractinian genera were frequently detected on shallow reefs, the genus *Seriatopora*, which is intolerant to heat stress, was more commonly found on deep reefs. This finding supports the hypothesis that deep coral reefs serve as thermal refugia.

【P-81】

イシサンゴ目を対象とした環境 DNA メタバーコーディング系の検討

Evaluation of eDNA metabarcoding assays for Scleractinian corals (order Scleractinia)

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近年、海水温の上昇に伴い造礁サンゴの分布は高緯度域へと拡大しており、その変化を効率的に把握するモニタリング手法の確立が求められている。本研究では、造礁サンゴの大部分を占めるイシサンゴ目を対象とした環境 DNA メタバーコーディング系の検出精度向上を目的とし、異なる遺伝子領域を標的として作製された 2 種類の既存プライマーの比較検証ならびに実験プロセスの検討を行った。実験プロセスの一つである PCR 酵素の検討では、特定の酵素は他の酵素と比較してより明瞭な増幅バンドが確認され、高い増幅性能を示した。今後、引き続き実験条件のさらなる最適化および既存プライマーの比較検証を進めることで、イシサンゴ目を対象とした環境 DNA メタバーコーディングの検出精度向上が期待される。本手法確立により、広域沿岸における造礁サンゴの分布変化を効率的かつ高精度に把握するモニタリング手法の実現を目指す。

In recent years, as sea temperatures have risen, the distribution of reef-building corals has expanded toward higher latitudes, creating a need to establish monitoring methods that can efficiently track these changes. In this study, with the aim of improving the detection accuracy of eDNA metabarcoding assays targeting Scleractinia, which constitute the majority of reef-building corals, we conducted a comparative evaluation of two existing primer sets for Scleractinia designed to target different gene regions and evaluated experimental procedures. In the evaluation of PCR polymerases, one of the experimental procedures, results suggested that a particular enzyme produced clearer amplification bands than the others, indicating superior amplification performance. Further optimization of experimental conditions and continued comparison of the existing primer sets are expected to improve the detection performance of eDNA metabarcoding for Scleractinia. detection accuracy of eDNA metabarcoding for Scleractinia. By establishing this method, we aim to develop a monitoring technique capable of efficiently and accurately tracking changes in the distribution of reef-building corals across extensive coastal areas.

Express yourself – Assessing taxonomic diversity and functional gene expression of subtropical shallow water coral reefs based on environmental DNA/RNA

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Environmental DNA (eDNA) metabarcoding has transformed biodiversity monitoring by providing non-invasive, sensitive community profiles, but DNA persistence might obscure short-term ecological signals. Environmental RNA (eRNA), in contrast, degrades more rapidly and may better capture present community states. This study evaluates the complementary roles of eDNA and eRNA for biodiversity assessment and wants to introduce environmental metatranscriptomics as a tool for diagnosing reef ecosystem health.

Water samples were collected across nine coral reef sites spanning a gradient of anthropogenic stress on Okinawa Hontō, Japan, at three points: April 2024, August 2024, and April/May 2025. The first time point captured the system before summer warming, the second coincided with the onset of severe visual bleaching in shallow reefs, and the third occurred one year later after high bleaching mortality. eDNA and eRNA were co-extracted, with eRNA reverse-transcribed to cDNA. Both templates were amplified using 18S rRNA gene markers to compare taxonomic resolution, community composition, and each method to detect spatial and temporal ecological differences. eRNA will additionally be sequenced to explore community-wide gene expression profiles, providing functional signatures that may reveal molecular stress responses and early indicators of ecosystem decline or resilience.

By integrating eDNA, eRNA, and metatranscriptomic approaches, this study aims to establish a framework for high-resolution biodiversity monitoring and ecosystem health assessment in reef environments. The findings will evaluate whether eRNA-based methods can complement eDNA in detecting active communities and diagnosing emerging stress in rapidly changing marine environments.

Herbivorous fishes are central hubs of a coral reef fish interaction network inferred from daily environmental DNA time series

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Herbivorous fish are widely regarded as important species in coral reefs because their intense grazing suppresses macroalgae, mediates coral–algal competition, and helps maintain reef resilience. They also engage in diverse biotic interactions including territorial defense and algal turf farming, thereby influencing other fishes. These roles have been demonstrated primarily through localized manipulations, such as exclusion of these fish, yet whether herbivorous species occupy central positions in the interaction network of a reef fish community has rarely been tested directly in the field. Here, we monitored daily fluctuations of fish communities using quantitative environmental DNA (eDNA) metabarcoding, together with various environmental variables, over 60 consecutive days at a shallow coral reef in Okinawa, Japan. We then applied empirical dynamic modelling (EDM), a nonlinear time-series approach, to infer the direction and dynamic strength of causal interactions among species while conditioning on environmental drivers, and quantified network metrics for each species. A territorial farming damselfish (*Pomacentrus chrysurus*) and a roving browsing surgeonfish (*Naso unicornis*) exhibited the highest hub centrality, indicating that these herbivores strongly influence other influential species and occupy central positions within the community network. At the whole network level, stronger turbulent mixing was associated with greater positive, negative, and overall interaction strength, suggesting that hydrodynamically energetic conditions intensify biotic coupling. Together, these findings show that ecologically important species can be identified directly from short-term behavioral dynamics captured by quantitative eDNA analyzed with EDM.

【P-84】

複数分類群を対象とした環境 DNA メタバーコーディングによるコアマモ場の生物相評価

Multi-taxon eDNA metabarcoding for assessing the faunal assemblages of a dwarf eelgrass bed

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海草藻場は、生物の生息・産卵・成育場としての機能に加え、水産資源の維持、生物多様性保全、ブルーカーボンによる気候変動対策の観点からも、その重要性が再認識されている。しかし、海域における環境 DNA 研究では魚類を対象とした現場適用が先行し、藻場生態系の重要な構成種である底生無脊椎動物を対象とした包括的な評価事例は限られている。

そこで本研究では、静岡県御前崎市の久々生海岸にあるコアマモ場と、隣接する岩礁帯において試料を採水し、魚類に加え、主要な底生無脊椎動物である軟体動物、環形動物、棘皮動物、節足動物（甲殻類）、刺胞動物を対象とした環境 DNA メタバーコーディング解析を行った。本研究では、分類群ごとの検出種数および種組成を比較することで、魚類のみを対象とした調査では把握困難な生物相を明らかにすることを目的とし、複数分類群の解析により得られた知見と、藻場の生物相評価手法としての有効性を報告する。

Seagrass beds are increasingly recognized not only as habitats, spawning grounds, and nursery grounds for marine organisms but also from the perspectives of sustaining fisheries resources, conserving biodiversity, and mitigating climate change through blue carbon. However, in marine environmental DNA research, field application has advanced primarily for fishes, and comprehensive assessments targeting benthic invertebrates, which are key components of seagrass bed ecosystems, remain limited.

In this study, we collected water samples from a dwarf eelgrass (*Zostera japonica*) bed on the Kubisho Coast, Omaezaki, Shizuoka Prefecture, Japan, and from an adjacent rocky reef, and conducted eDNA metabarcoding analyses targeting fishes as well as the major benthic invertebrate groups: molluscs, annelids, echinoderms, arthropods (crustaceans), and cnidarians. By comparing the number of detected taxa and the species composition among taxonomic groups, we aimed to characterize elements of the biota that are difficult to detect in fish-only surveys. Here we report the findings obtained from this multi-taxon analysis and discuss its utility as a method for assessing the biota of seagrass beds.

【P-85】

環境 DNA からみた小河川汽水域および接続する塩性湿地における魚類の検出状況

Fish Detection Using Environmental DNA in a Small River Estuary and Its Adjacent Salt Marsh

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宮城県気仙沼市の西舞根川は、全長 1.8km、川幅約 3 m、汽水域長約 200 m の小河川で、汽水域には面積約 9,000 m²の塩性湿地が接続している。河川では河口から上流にかけて環境条件が変化し、魚類の利用環境も変化するが、小河川汽水域における魚類の検出種数に関する知見は限られる。そこで、年に 4 回、干潮時に採水を行い、西舞根川と同河川に接続する塩性湿地を対象とした環境 DNA メタバーコーディングにより魚類の検出種数を把握した。

河川ではチチブ属をはじめとして 28 ～ 62 種、湿地ではボラをはじめとする 14 ～ 30 種が検出された。既往研究では、汽水域長約 3 km の河川で 40 ～ 56 種、約 150 km の河川で 73 種が検出されており、本研究の小規模な汽水域でも同程度の魚種数が検出された。これより、小規模河川の汽水域も多様な魚類が利用する重要な環境となりうる可能性が示唆された。

The Nishi-Moune River is a small river, approximately 2.5 m wide and 2 km long, located in Kesennuma City, Miyagi Prefecture. Saltwater intrudes up to 0.3 km upstream from the river mouth, and a salt marsh of approximately 9,000 m² is connected to the river within this brackish zone.

Although environmental conditions and fish habitats vary considerably along rivers from upstream reaches to the estuaries, research on fish species occurrence in small river estuaries remains limited. In this study, fish species were examined at low tide using environmental DNA (eDNA) metabarcoding analysis four times over one year.

A total of 28–62 fish species, including *Tridentiger obscurus*, were detected in the river, while 14–30 species, including *Plecoglossus altivelis*, were detected in the adjacent salt marsh.

Previous studies reported 40–56 species in a river with a 3 km long estuary and 73 species in a river with a 150 km long estuary. Despite its much smaller spatial scale, the Nishi-Moune River estuary showed a comparable number of detected fish species.

These results suggest that even small river estuaries and their adjacent salt marshes may provide important habitats for diverse fish species.

【P-86】

沿岸海域および河川汽水域におけるキチヌとクロダイの環境 DNA 分布の比較

Comparison of environmental DNA distributions of yellowfin sea bream and black sea bream in coastal waters and riverine brackish waters

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同所分布するキチヌとクロダイはともに海から汽水域までを利用する広塩性魚類である。本研究では、両種の eDNA 分布を海および河川汽水域で比較し、両種の環境利用の違いを調べることを目的とした。

2026 年春から夏にかけて、広島県呉市の黒瀬川と周辺沿岸海域（海）の各 3 定点で毎月環境水を採集した。両種それぞれの種特異的検出系による定量 PCR により eDNA コピー数を定量した。クロダイの eDNA コピー数に対するキチヌの相対量（キチヌ相対量）を河川と海で比較するとともに、各定点における両種の eDNA コピー数を比較し、その水平分布を調べた。

河川では海よりキチヌ相対量が高かった。河川内でキチヌの eDNA コピー数は河口域で高く、反対にクロダイでは塩水遡上部の上端付近で eDNA コピー数が高い傾向がみられた。キチヌは河口域周辺を中心に利用する一方、クロダイは塩水の遡上とともに河川内へ進入している可能性がある。

The yellowfin seabream *Acanthopagrus latus* and black seabream *A. schlegelii* are euryhaline fishes that occur sympatrically and use habitats ranging from coastal marine waters to estuaries. This study aimed to examine differences in habitat use between the two species in coastal marine and estuarine environments by comparing their species-specific eDNA distributions.

Water samples were collected monthly from spring to summer 2026 at three sampling sites in the Kurose River and three sites in the adjacent coastal waters of Kure, Hiroshima, Japan. The eDNA copy number of each species was quantified by qPCR using species-specific detection assays. We compared the relative eDNA signal of yellowfin seabream to black seabream between the river and coastal waters. We also compared the eDNA copy numbers of both species among sampling sites to examine their horizontal distributions.

The relative eDNA signal of yellowfin seabream was higher in the river than in the coastal waters. Within the river, yellowfin seabream eDNA copy numbers were highest near the river mouth, whereas those of black seabream tended to be highest near the upstream limit of saltwater intrusion. These results suggest that yellowfin seabream primarily use areas around the river mouth, whereas black seabream may extend their distribution into the river in association with upstream saltwater intrusion.

【P-87】

養殖海藻が固定した炭素の海底への隔離を DNA で探る

Exploring the seafloor sequestration of carbon fixed by farmed seaweeds using seaweed-derived DNA

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養殖海藻が固定した炭素の一部は、藻体や多糖などの有機物として海域に放出される。海藻由来の有機物が海域の底質に長期間残存する場合、海藻養殖は大気中の二酸化炭素の隔離に寄与すると考えられる。そこで本研究では、養殖海藻由来有機物の海底堆積物中の残存を検討する目的で、主要養殖種であるオキナワモズク（以下モズク）の養殖海域およびその周辺海域の堆積物コアに含まれるモズク DNA を定量した。堆積物コアは沖縄県与勝海域において採取し、モズク DNA は我々が開発したデジタル PCR 法によるモズク DNA 特異的検出系により定量した。その結果、養殖海域の堆積物コアでは周辺海域と比べてモズク DNA 濃度が高く、またコア上層部では下層部より高かった。モズク DNA が残存する堆積物にはより難分解性の他のモズク由来有機物も残存する可能性があることから、モズク由来有機物の一部は海底に一定期間残存すると考えられた。

Seaweeds release a portion of their fixed carbon into surrounding waters as detached biomass and organic compounds, such as polysaccharides. The long-term retention of organic carbon derived from seaweed aquaculture in marine sediments plays a role in sequestering atmospheric carbon dioxide. To investigate the persistence of organic matter derived from cultivated seaweeds in marine sediments, we focused on DNA from *Cladosiphon okamuranus* (Okinawa-mozuku, a major cultivated seaweed species) contained in sediment cores collected from a seaweed farm and adjacent sites in the Yokatsu area of Okinawa, Japan. The concentrations of *C. okamuranus* DNA within these cores were quantified using digital PCR with species-specific primers and a probe developed by our research group. Results indicated that *C. okamuranus* DNA concentrations were higher in cores from the farm site compared to the nearby control sites and were particularly elevated in the upper sediment layers. These findings suggest that organic matter originating from *C. okamuranus*, especially compounds that are more recalcitrant than DNA, can persist in marine sediments over extended periods.

A physiological perspective on environmental RNA and fish reproductive behaviors

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Environmental RNA (eRNA) detection and assessment is a growing theme in ecology as it can potentially indicate functional aspects of organisms in the wild by non-invasive means. eRNA studies complement those using environmental DNA (eDNA) where species identity is primarily targeted. In a scenario where fish reproduce, large quantities of sperm and eggs are released into the environment, transiently increasing the gamete-specific RNA content in the water. Our group previously showed that a testis-specific eRNA marker (*klhl10*) was transiently increased in the water when mating behavior was prominent in medaka. This study used an eRNA sequencing (eRNAseq) approach to identify novel reproductive behavioral eRNA markers that can be robustly measured. Using differential gene expression analysis, 42 genes were found to have higher transcript abundance in the water where mating behavior was prominent. Among these candidate genes, glutamic-acid rich protein-like (*garp*-like) and polyamine modulated factor 1 binding protein 1-like (*pmfbp1*-like) were abundantly expressed in the testis. The transcript abundance of *pmfbp1*-like was positively correlated to that of *klhl10* and was a thousand-fold more abundant than *klhl10*, thereby supporting its use as a robust reproductive behavioral eRNA marker. Using *in situ* hybridization chain reaction, the localizations of *garp*-like, *pmfbp1*-like, and *klhl10* were examined in the testis, and we showed that *garp*-like and *pmfbp1*-like were expressed by elongating and elongated spermatozoa while *klhl10* was abundantly expressed at round spermatozoa stage. Our data support the use of eRNAseq in the search for eRNA markers related to specific physiological phenomena including reproduction, stresses, and diseases.

【P-89】

PMA を用いたアユ配偶子由来 eDNA の選択的検出と河川への適用検討

Selective Detection of Gamete-Derived eDNA of Ayu (*Plecoglossus altivelis altivelis*) Using PMA (Propidium monoazide)

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配偶子に由来する環境 DNA (eDNA) の検出は魚類の産卵行動の把握に有用である。本研究では、Propidium Monoazide (PMA) 処理後に検出される細胞内 DNA を配偶子 (精子) の指標として利用し、アユ (*Plecoglossus altivelis*) への適用性を検討した。精子添加試験では、アユの飼育水および精子添加飼育水を 14、8、2、0.6 μ m フィルターで粒径分画し、PMA 処理の有無を比較した。その結果、飼育水では 2 μ m 未満画分の細胞内 DNA 割合が低下し、精子添加区では増加する傾向が認められた。さらに、滋賀県姉川および知内川の産卵期調査では、有効産卵数の増加に伴い 2 μ m 未満画分の細胞内 DNA 割合が増加する傾向が認められた。以上より、PMA 処理と粒径分画を組み合わせることで、配偶子を含む細胞内 DNA を指標としたアユの産卵モニタリングが可能となることが示唆された。

Detection of gamete-derived environmental DNA (eDNA) is a promising approach for monitoring fish spawning activity. In this study, we evaluated the applicability of PMA-derived intracellular DNA as a proxy for gametes in ayu (*Plecoglossus altivelis*). Laboratory experiments were conducted using three types of water samples: water, tank water collected from ayu rearing tanks, and the same tank water supplemented with ayu sperm. Samples were sequentially filtered through 14, 8, 2, and 0.6 μ m filters, and eDNA was analyzed with and without Propidium Monoazide (PMA) treatment. In water samples collected from ayu rearing tanks, the proportion of intracellular DNA in the 2 μ m fraction tended to decrease, whereas the addition of ayu sperm increased this proportion. Field surveys conducted during the spawning season in the Ane and Chinai rivers, Shiga Prefecture, showed that the proportion of intracellular DNA in the 2 μ m fraction was positively associated with the number of eggs visually observed during spawning surveys. These results suggest that PMA-derived intracellular DNA can serve as a proxy for gametes, enabling monitoring of ayu spawning activity when combined with size-fractionated filtration.

【P-90】

環境エクソソーム /sEVs (eExosomes/esEVs) 由来 RNA によるワカサギ産卵イベントの検出

Detection of pond smelt spawning events using RNAs derived from environmental exosomes/sEVs (eExosomes/esEVs)

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水圏生物が環境水中に放出するエクソソーム / 小型細胞外小胞 (sEVs) を回収・解析する手法を開発した (Yonezawa et al., *Mar Biotechnol*, 2026)。本研究では本手法の集団産卵モニタリングへの応用を目指し、環境刺激により水槽内自然産卵が可能なワカサギを対象とした。芦ノ湖で漁獲した個体を水槽で産卵誘導し、誘導前・産卵中・1日後の水槽水から限外濾過によりエクソソーム /sEVs 画分を回収して mRNA 解析を行った。ゲノムマッピング率は 0.5-7.9% と既存の環境 RNA 解析と同等以上であった。産卵中にはミトコンドリアおよび精子・鞭毛関連遺伝子が、1日後には筋肉・解糖系・卵膜関連遺伝子が高く、産卵に伴う組成変化が示唆された。現在、エクソソーム /sEVs に特徴的な small RNA 解析も進めており、環境エクソソームを用いた産卵モニタリングの可能性を議論する。

Aquatic organisms release exosomes and small extracellular vesicles (sEVs) into the surrounding water, and we recently established a method to recover and analyze these environmental exosomes/sEVs (eExosomes/esEVs; Yonezawa et al., *Mar Biotechnol*, 2026). Here we examined whether this approach can monitor mass spawning events. We selected the pond smelt (*Hypomesus nipponensis*), which can be induced to spawn naturally in tanks by environmental stimuli. Fish captured in Lake Ashinoko were maintained in a 1.5-t tank and induced to spawn under shading. Tank water was sampled before induction, during spawning, and one day after spawning. After filtration through 0.22 μ m, the eExosome/esEV fraction was recovered by tangential flow filtration followed by ultrafiltration (100 kDa), and RNA extracted from the fraction was subjected to mRNA sequencing. Mapping rates against the reference genome were 0.5-7.9%, comparable to or higher than those of conventional eRNA analyses. During spawning, mitochondrial genes and candidate sperm- and flagella-related genes were detected, suggesting a contribution of sperm-derived RNAs or particles to the water fraction. One day after spawning, muscle, glycolysis, and egg envelope/proteolysis-related genes were enriched, indicating a compositional shift of the eExosome/esEV fraction. Because exosomes/sEVs are characteristically enriched in small RNAs, we are currently performing small RNA sequencing of the same fractions to obtain vesicle-derived signatures that are less affected by free RNA. We discuss the potential of eExosome/esEV analysis as a non-invasive tool for monitoring reproductive events in aquatic organisms.

【P-91】

ホトケドジョウ (*Lefua echigonia*) を対象とした eRNA 分析：水槽実験による手法最適化と現地検証

eRNA Analysis for *Lefua echigonia*: Tank Optimization and Field Validation

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環境 RNA (eRNA) は生物の生体活動を直接反映することができると考えられている。しかし eRNA を用いた生物調査の知見は限られている。そこでホトケドジョウを用いて、種特異的な環境 RNA 調査手法の検討を行った。まず野外でホトケドジョウの現地調査を実施した結果、eDNA は十分量検出された一方、eRNA の値は低かった。そこで分析フローの再検討と最適化を行った。具体的には、ホトケドジョウが飼育されている水槽水をろ過したフィルターからの① eRNA 抽出工程ならびに②逆転写のステップについて改良を行い、最終的な収量について初回法と改良法を比較した。その結果、改良法では eRNA 収量の大幅な改善が示された。野外で改良法により再度現地調査を行ったところ、eRNA 収量は大幅に増加した。以上より、分析フロー全体の最適化により eRNA 収量を改善し、より高感度で再現性の高いモニタリングが可能であることが示された。

Environmental RNA (eRNA) degrades faster than environmental DNA (eDNA) and is therefore less likely than eDNA to be affected by residual signals from carcasses, allowing it to more dynamically reflect the biological activity of target organisms. However, knowledge of its application in biological surveys remains limited. In this study, we evaluated whether species-specific eRNA could refine the distribution of the *Lefua echigonia*, a conservation-priority species that is relatively difficult to detect using conventional capture surveys and examined the associated methodology. Initial field monitoring of *L. echigonia* detected sufficient eDNA but yielded low eRNA levels, prompting a review and optimization of the analytical workflow. Using Sterivex cartridges prepared by filtering water from tanks containing *L. echigonia*, we optimized the eRNA extraction and reverse transcription steps and compared the final eRNA yields between the original and optimized methods. The optimized methods substantially increased eRNA recovery. When the optimized method was applied to repeat surveys at the same field site, eRNA levels relative to eDNA increased 1.49–2.83-fold after optimization. These results demonstrate that systematic optimization of the entire analytical workflow can improve eRNA recovery and support more sensitive and reproducible eRNA monitoring.

【P-92】

ポリエステルウールを用いた環境 RNA 回収によるヤイトハタの時計遺伝子 *per2* 日周発現の検出

Polyester wool-based environmental RNA collection enables detection of diel *per2* expression in the Malabar grouper (*Epinephelus malabaricus*)

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環境 RNA (eRNA) 解析は、水生生物を非侵襲的にモニタリングできる手法として注目されているが、従来法はろ過操作に依存し、養殖現場での利用に課題がある。本研究では、ポリエステルウールを用いた簡便な eRNA 回収法を開発し、ヤイトハタ (*Epinephelus malabaricus*) を用いて評価した。飼育水から回収した eRNA では、ハウスキーピング遺伝子および時計遺伝子 *per2* の転写産物が検出され、魚の除去後も最大 8 時間検出可能であった。また、eRNA 由来の *per2* 日周発現パターンは筋肉、下垂体、肝臓の発現動態と概ね一致した。これらの結果は、本手法が養殖環境における時計遺伝子の日周発現を非侵襲的に評価する手法として有望であることを示した。

Environmental RNA (eRNA) analysis has attracted increasing attention as a non-invasive approach for monitoring aquatic organisms because it can provide information on actively expressed genes. However, many existing eRNA sampling methods rely on filtration-based procedures that require specialized materials and substantial sample processing, limiting their practical application in aquaculture settings. In this study, we developed a simple polyester wool-based method for collecting eRNA directly from fish rearing tanks. Using the Malabar grouper (*Epinephelus malabaricus*) as a model species, we conducted three experiments to evaluate the detectability, persistence, and temporal expression patterns of eRNA collected using this approach. eRNA obtained from rearing water contained detectable transcripts of housekeeping genes and the circadian clock gene *per2*. In a closed recirculating system, the target transcript remained detectable for up to 8 h after fish removal. Furthermore, diel expression patterns of *per2* detected in rearing-water eRNA were broadly similar to those observed in muscle, pituitary, and liver tissues. Heatmap visualization, cross-correlation analysis, and cosinor analysis suggested temporal similarity between aqueous eRNA signals and tissue expression profiles. These findings demonstrate the feasibility of detecting diel clock gene expression using polyester wool-based eRNA collection. The proposed approach provides a simple and non-invasive method for eRNA sampling in aquaculture systems, although further validation across additional genes, species, and culture conditions is required.

【P-93】

自動採水装置による連続モニタリングと環境 DNA メチル化解析に基づく カワバタモロコの野外産卵条件の解明

Continuous Monitoring with Automated Water Samplers and Environmental DNA Methylation Analysis Reveal the Field Spawning Conditions of *Hemigrammocypris neglecta*

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環境 DNA 分析は主に DNA 配列に基づく生物分布の把握に用いられてきたが、近年は DNA メチル化を利用して生理状態を推定する技術が注目されている。しかし環境 DNA メチル化解析は、これまで水槽実験や管理されたビオトープなど、限られた条件下での検証にとどまっている。

本研究では、自然分布域の3つの生息池で8日間、開発した自動採水装置による定期採水し、魚類の精子で高メチル化する DNA マーカーを用いてカワバタモロコの産卵を検出を行った。その結果、カワバタモロコの産卵は、降雨に伴う水温の低下または水位の上昇と関連していることが示された。また、調査期間中に実施した3時間間隔の連続モニタリングにより、本種の産卵は、これまで報告されていた日中だけでなく、深夜帯にも活発に行われることが明らかとなった。さらに、これらの結果に基づいて夜間観察を実施し、調査地におけるカワバタモロコの産卵行動を撮影することに成功した。

Environmental DNA (eDNA) analysis has primarily been used to determine the distribution of organisms based on DNA sequence information. Recently, methods that use DNA methylation to infer the physiological states have attracted increasing attention. However, eDNA methylation analysis has so far been validated only under limited conditions, such as tank experiments and managed biotopes.

In this study, we detected spawning by the golden venus chub (*Hemigrammocypris neglecta*) using a DNA marker specifically hypermethylated in fish sperm and conducted periodic water sampling with automated water samplers developed for this study over eight days in three ponds within the species' natural range. The results indicated that spawning by *H. neglecta* was associated with rainfall-induced decreases in water temperature or increases in water level. In addition, continuous monitoring at 3-h intervals revealed that spawning occurred actively not only during the daytime, as previously reported, but also around midnight. Based on these findings, we conducted nighttime observations and successfully recorded the spawning behavior of *H. neglecta* at the study sites.

DNA Methylation-Based Age Estimation for Cetaceans in Hong Kong waters Using Methylation-Sensitive High-Resolution Melting Analysis

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Age estimation is essential for understanding cetacean population dynamics and conservation management, as age structure influences reproductive potential, survival, physical and mental welfare and ultimately population recovery capacity, but traditional growth layer group (GLG) methods require tooth extraction and are mainly applicable to stranded or deceased individuals. Here, we developed the first DNA methylation epigenetic clock for Phocoenidae, the Indo-Pacific finless porpoise (*Neophocaena phocaenoides*), using an optimized methylation-sensitive high-resolution melting (MS-HRM) workflow. Chronological ages were determined by GLG analysis for 28 stranded individuals, and methylation levels of three age-associated loci, *GRIA2*, *CDKN2A*, and *TET2*, were quantified from tissue-derived (muscle and liver) DNA. Although individual loci showed limited age associations, the combined methylation profiles were successfully integrated using support vector regression (SVR). The optimal model showed reliable predictive performance under leave-one-out cross-validation (LOOCV), with a mean absolute error of 1.35 years and an R^2 of 0.620. This model was further applied to 16 stranded individuals with unknown age, whose predicted ages ranged from 7.75 to 18.27 years. This study demonstrates that conserved cetacean age-associated methylation markers can be applied to Phocoenidae species and highlights the potential universality of *GRIA2*, *CDKN2A*, and *TET2* for cetacean epigenetic clocks. Furthermore, the successful application to highly decomposed stranded samples provides a practical approach for cetacean age assessment and conservation studies.

環境 RNA/DNA の生分解性情報に基づく偽陽性核酸の分布予測

Predicting the distribution of false-positive nucleic acids using biodegradation parameters of environmental RNA/DNA

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環境 RNA/DNA (eRNA/eDNA) メタバーコーディング解析は、水環境の生物群集を推定する有望なモニタリング技術であるが、生活排水に由来する偽陽性核酸を検出することが課題である。我々は、生活排水中の魚類核酸量や eRNA と eDNA の比率、偽陽性核酸の生分解性情報をパラメーターとした分布予測モデリングにより、偽陽性核酸による汚染状況を可視化できると考えた。本研究では、産総研一水系暴露解析モデルを用いて、人口や土地利用などのモデル内蔵データに加え、下水処理場での偽陽性核酸存在量および水槽実験から取得した生分解性情報を用いて偽陽性核酸の時空間的分布状況を予測した。利根川水系では偽陽性 eDNA による汚染が広範囲に及ぶ一方、偽陽性 eRNA の汚染範囲は相対的に小さいことが示された。本手法による偽陽性核酸の分布予測は、偽陽性リスクの事前評価だけでなく、解析結果の補正や調査設計の判断にも役立つ。

Environmental RNA/DNA (eRNA/eDNA) metabarcoding is a promising monitoring approach for estimating biological communities in aquatic environments; however, false-positive signals derived from domestic wastewater remain a major challenge. We hypothesized that contamination by such false-positive nucleic acids could be visualized through distribution-prediction modeling parameterized with (i) the amount of fish nucleic acids in domestic wastewater, (ii) the ratio of eRNA:eDNA in domestic wastewater, and (iii) the biodegradability of eRNA/DNA, including removal rate in wastewater treatment plants (WWTPs) and half-lives in aquatic environments.

In this study, the national institute of Advanced Industrial Science and Technology-Standardized Hydrology-based Assessment tool for chemical Exposure Load (AIST-SHANEL) was applied to predict the spatiotemporal distribution of false-positive nucleic acids by combining built-in datasets (e.g., population, land use, and elevation) with obtained experimental parameters, namely the ratio of false-positive eRNA/eDNA in domestic wastewater, the removal rate in WWTPs and half-lives. Model outputs showed that false-positive eDNA contamination is widespread in the Tone River basin, whereas the contamination range associated with false-positive eRNA is comparatively limited.

These predictions can facilitate not only preliminary assessments of false-positive risk at survey sites, but also practical decisions on whether and how to correct metabarcoding results and how to design field surveys (e.g., selecting eDNA versus eRNA based on expected domestic wastewater influence).

【P-96】

多検体処理に向けた環境 DNA 分析手法の検討：学会マニュアルとの比較

Toward high-throughput eDNA analysis: a comparison with the eDNA Society standard manual

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2026 年度より、河川水辺の国勢調査において環境 DNA 調査が正式に採用された。洋上風力発電施設等の設置に伴う海域の環境調査においても、環境 DNA の活用が期待される。今後、環境 DNA 調査の需要が急速に高まり、多数の検体を正確かつ効率的に分析する必要が生じると見込まれる。現状の分析手法は環境 DNA 学会が公開するマニュアル（以下、学会マニュアル）が基本となるが、これは汎用性を重視して策定されており、分析の自動化など多検体処理を想定した手法とはなっていない。

我々は学会マニュアルで採用されているカラム式 DNA 抽出法に対し、磁気ビーズ式抽出法を導入することで多検体処理への対応を可能とした。さらに、ライブラリ調製工程に自動分注機を導入することで、精度の担保と省力化の両立を図った。本発表では、分析精度を確保しつつ多検体を効率的に分析する手法を検討し、学会マニュアル準拠の手法との比較検証の結果を報告する。

In fiscal year 2026, environmental DNA (eDNA) surveys were formally adopted for the National Census on River and Dam Environments. The use of eDNA is also anticipated in marine environmental surveys associated with the installation of offshore wind power facilities and similar projects. Domestic demand for eDNA surveys is therefore expected to rise rapidly, creating a need to analyse large numbers of samples both accurately and efficiently. Current analytical work is based primarily on the manual published by the eDNA Society (hereafter, the manual); however, this manual was formulated with an emphasis on general applicability and does not provide methods designed for high-throughput processing, such as automated analysis.

In this study, we replaced the column-based DNA extraction method used in the manual with a magnetic-bead-based method, thereby enabling high-throughput sample processing. In addition, we introduced an automated liquid handler into the library preparation step to reconcile assured precision with reduced labour. Here we examine a workflow for analysing large numbers of samples efficiently while maintaining analytical accuracy, and report the results of a comparative validation against the procedure compliant with the manual.

【P-97】

パッシブサンプリング法による最適な設置時間の検討

Investigation of the optimal deployment duration for Passive eDNA Sampling using natural dried marine sponges

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近年、捕集材を一定期間環境中に曝露することで環境 DNA を捕集するパッシブサンプリング法（PS 法）が新たな環境 DNA 調査手法として注目されており、海外を中心に研究事例が増加している。PS 法は環境 DNA を時間積算的に捕集できるため、採水法では把握しにくい低密度種や夜行性種の検出効率向上が期待される。一方で、DNA の蓄積過程や最適な設置時間に関する知見は十分ではなく、標準化に向けた課題となっている。本研究では、天然乾燥海綿を捕集材として用い、設置時間に着目した実験を実施した。現在の運用では一昼夜程度の設置を目安としており、今回は、設置後 3 時間までは 1 時間ごと、その後は 3 時間ごとに回収し、最大 24 時間まで曝露した。設置時間が種組成および種ごとの DNA 量に及ぼす影響を評価し、その結果をもとに目的に応じた適切な設置時間について考察する。

In recent years, passive environmental DNA (eDNA) sampling, which collects eDNA by exposing sampling materials to aquatic environments for a certain period, has attracted increasing attention as a novel eDNA monitoring method, and the number of studies has been increasing, particularly overseas. Because passive sampling enables the time-integrated collection of eDNA, it is expected to improve the detection of low-density and nocturnal species that are often difficult to detect using conventional water sampling methods. However, the processes of eDNA accumulation and the optimal deployment duration remain insufficiently understood, posing challenges for the standardization of this method.

In this study, natural dried marine sponges were used as sampling materials to investigate the effects of deployment duration. Under the current protocol, samplers are typically deployed for approximately one day. In this experiment, samplers were retrieved at 1-h intervals during the first 3 h after deployment and subsequently at 3-h intervals, with a maximum deployment time of 24 h. We evaluated the effects of deployment duration on species composition and species-specific eDNA quantities. Based on the results, we discuss the appropriate deployment duration according to monitoring objectives when using natural dried marine sponges for passive eDNA sampling.

【P-98】

パッシブサンプラーの最適用法：理論解析による DNA 蓄積量予測

Optimizing passive eDNA sampling: Theoretical framework for time-dependent eDNA and retrieval

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パッシブ eDNA サンプリングは設置期間中の DNA シグナルを蓄積するが、設置時間と検出可能な DNA 量の関係はよく分かっていない。本研究では、DNA 供給、分解、基質容量を組み込んだ理論モデルを構築し、その予測を試みた。分解 DNA が基質容量を占有しない場合、検出可能な DNA 量は単調に増加したが、その平衡量は供給率に比例せず、eDNA 供給量を過小評価する結果となった。一方、分解 DNA が基質を占有し続ける場合、検出可能な DNA はピーク後に減少した。高い eDNA 供給率はピーク量を増加させる一方でピークを早め、長時間設置では eDNA 高供給条件が低供給条件より低い検出量を示す可能性が生じることが明らかとなった。このため、サンプラーの回収時間を最適化するには、基質容量のみならず、基質上での DNA 分解率および分解 DNA による基質占有率を考慮する必要がある。

Passive eDNA sampling integrates DNA signals over deployment time, but how deployment duration affects the amount of detectable DNA retained by a sampler remains poorly understood. In this study, a theoretical model was developed to examine how DNA input, degradation, and finite substrate capacity shape eDNA accumulation. The model assumed a constant DNA input during the sampler deployment, and partial DNA degraded on the substrate remaining on the sampler and occupying its capacity. Major findings of this study were; (1) when degraded DNA does not occupy substrate capacity, the sampler accumulated DNA monotonically, but the equilibrium amount of detectable eDNA increased less than proportionally with input rate; (2) When degraded DNA continued to occupy substrate capacity, detectable DNA increased to a peak and subsequently declined. In the latter case, higher DNA input rates increased peak yield, but caused the peak to occur earlier. In contrast, greater substrate capacity increased peak yield and delayed the peak. Consequently, the yield-maximizing retrieval time was parameter dependent, and prolonged deployment could cause a higher-input condition to yield less detectable eDNA than a lower-input condition. The model predicts that passive sampling can generate either saturating or unimodal accumulation dynamics, distort proportional differences in environmental DNA supply, and even reverse the rank order among input conditions. Retrieval timing, substrate capacity, and the relationship between DNA detectability and substrate occupancy should therefore be explicitly considered in the design and quantitative interpretation of passive eDNA surveys.

【P-99】

環境 DNA パッシブサンプリング法におけるサンプル保存方法に関する比較検討

Comparison of sample preservation strategies for environmental DNA passive sampling method

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近年、環境 DNA 調査手法のひとつとして、DNA 吸着素材を水中に長時間浸漬するパッシブサンプリング法 (PS 法) が提案されている。本研究では、PS 法における最適なサンプル保管方法の探索を目的として、パッシブサンプルに添加する保存試薬および保存期間の違いが検出魚種数に与える影響を比較した。河川に 24 時間浸漬したパッシブサンプルを室温 (RNA later、オスバン、エタノール、添加なし) と冷凍の 5 条件で処理し、それぞれ 3 種類の保存期間 (24 時間、48 時間、72 時間) で比較した。その結果、すべての処理条件および保存期間において、検出魚種数および種組成に有意な違いはみられず、室温保管でも冷凍保管と同等の魚類多様性を検出できることが示唆された。一方で、常温・添加なし条件では DNA 濃度が大きく減少したことから、いずれかの試薬を添加することで、より安定した DNA の保存が可能になることが示された。

Passive sampling method (PS method) by submerging DNA adsorption materials in water has been developed as one of the environmental DNA surveys. In this study, with the aim of investigating the optimal sample preservation strategy for the PS method, we compared the effects of different solutions added to passive samples and different storage periods on the fish diversity detection. Passive samples submerged in river water for 24 hours were stored under the combination of five conditions—room temperature (RNA later, Osban S, ethanol, and additive-free) and frozen—and three storage durations (24 hours, 48 hours, and 72 hours). As a result, no significant differences in fish diversity were observed across all combinations of treatment conditions and storage durations. On the other hand, since the DNA concentration decreased significantly under the room temperature and additive-free condition.

Air Sampling and Sample Preparation Strategies for Highly Sensitive Environmental DNA Detection

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環境 DNA の検出に対する関心が高まっている中、我々は、環境や個体への侵襲を必要としない検体採集法としてエアーサンプリングを検討している。エアーサンプリングは非侵襲、かつ、広範囲の情報を見逃しなく一括して収集でき、また、自動捕集器の設置により、省力化の達成や経時変化の追跡も容易になることが期待される。しかし、他の検体採集法に比べてサンプル中の標的濃度は格段に希薄である点が課題であり、高感度検出プロトコルの開発と相まって、検体の濃縮技術の確立も極めて重要なポイントとなる。今回、我々は、ノニオン性界面活性剤の微量添加（容器やピペットチップなどへの非特異吸着ロス）やサンプルの濃縮（分散媒除去による標的 DNA の実効濃度増大）を施すことによって、分析プロセス全体として少なくとも数千倍の実効的な検出信号の増強を達成し、実質的な検出下限濃度を 3 桁低減できることを確認したので報告する。

With growing interest in environmental DNA (eDNA) detection, we are investigating air sampling as a non-invasive sampling method. Air sampling enables comprehensive, wide-area monitoring and can facilitate automated, continuous sample collection. However, target concentrations in air samples are substantially lower than with other sampling methods, making effective sample concentration essential. Here, we report that trace supplementation with a nonionic surfactant and sample concentration enhanced the effective detection signal by at least several thousand-fold. Consequently, the practical detection limit was reduced by approximately three orders of magnitude, demonstrating the potential of this approach for highly sensitive eDNA detection from air samples.

【P-101】

環境 DNA 分析における再現性と手法間の結果変動についての検討

Reproducibility and Variation in Results Among Methods in Environmental DNA Analysis

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フィールドで採取された環境 DNA はたいていの場合、濃度が薄く回収量も限られ、凍結融解や長期保管による劣化等もあるため、結果の再現性について明示的に議論することはあまりないようである。しかし、分析精度を考える上では、分析結果あるいは採取毎の再現性の議論はたいへん重要である。また、特にメタバーコーディング法の場合、分析手順の変更でデータがどのように変動するかは、分析結果を解釈する上で重要な要素であり、様々なデータを比較検討する際には、議論しておく必要がある。研究手法の規格化、標準化へと向かい、確立されたものとして議論されつつある現在の流れの中で、これらの基礎的な部分を再度見直すことは意義があると思う。

ここでは、同一条件で採取した試料、異なる条件で採取した試料を、いくつかの手法で分析を行い、結果を比較することで、その変動について検討したい。

Environmental DNA collected in the field is typically present at low concentrations and in limited quantities; furthermore, it is susceptible to degradation caused by factors such as freeze-thaw cycles and long-term storage. Consequently, the reproducibility of results is rarely explicitly discussed. However, addressing reproducibility—whether regarding the analytical results themselves or the consistency across sampling events—is crucial when considering analytical precision. This is particularly important for metabarcoding, where understanding how data fluctuates in response to changes in analytical procedures is vital for interpreting results and comparing various datasets. As the field moves toward the standardization of research methodologies and establishes accepted protocols, it is meaningful to re-examine these fundamental aspects. In this presentation, we aim to investigate data variability by analyzing samples collected under both identical and differing conditions using various methods and comparing the resulting data.

【P-102】

IPC の PCR 効率を用いた河川における分析阻害の見える化

Visualization of Mapping PCR Inhibition in Rivers Using IPC Amplification Efficiency

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環境 DNA 分析では、サンプル中に含まれる DNA 以外の物質が PCR の増幅効率に影響を与え、良好なデータが得られなくなる場合がある。実務の現場では、分析不調は業務の円滑な進行の妨げとなるだけでなく、本来いるはずの生物情報の欠損につながる恐れもある。分析阻害を引き起こす原因物質は多岐にわたることから、本研究では、分析不調の出現傾向について考察するための情報基盤として IPC の PCR 効率を指標に分析阻害レベルの見える化を行った。

In environmental DNA analysis, substances present in environmental samples other than DNA can inhibit PCR amplification, compromising the reliability of analytical results. In practice, PCR inhibition can hinder routine analyses, resulting in the loss of biological information or false-negative detections. Because the substances responsible for PCR inhibition are diverse, this study assessed and visualized inhibition levels in river samples using the amplification efficiency of an internal positive control (IPC; Nippon Gene) as indicators. Spatial and Seasonal patterns of PCR inhibition were then examined across the rivers.

【P-103】

環境 DNA の濃縮における従来濾過法と新規高効率濃縮法の比較評価

Comparison of conventional filtration and a novel high-efficiency concentration method for eDNA analysis

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環境 DNA を濃縮する際、開放型または閉鎖型のフィルターを用いる手法が代表的である。これらは環境水の状態や現場での運用条件などに応じて使い分けられている。近年、環境 DNA 濃縮の新技术としてガラス繊維を攪拌する QuickConc シリーズが開発され、その有用性が期待されているが、従来法との比較検証が十分ではない。本研究では河川、湖沼および海域といった水質の異なる複数の環境水をそれぞれ開放型の GF/F フィルター、閉鎖型の Sterivex フィルター、QuickConc Vacuum Plus の 3 通りの手法で濃縮し、その濃縮効率などを比較した。得られた結果をもとに様々な環境条件や分析条件に適した濾過法について議論する。

To concentrate eDNA, methods using open-type or closed-type filters are the most common. These have been selected based on the condition of the environmental water and field operational conditions. In recent years, the QuickConc series, which agitates glass fibers, has been developed as a new method for eDNA concentration. While it is expected to be useful, it has not yet been sufficiently validated through comparison with conventional methods. In this study, environmental water samples of various water types such as rivers, ponds, and marine waters were concentrated using three different methods, open-type GF/F filters, closed-type Sterivex filters, and QuickConc Vacuum Plus, and their concentration efficiencies were compared. We discuss which filtration method is suitable for various environmental and analytical conditions.

【P-104】

Nanopore を用いた環境 DNA 解析の精度面での利点

Advantages of Nanopore Sequencing for Improving the Accuracy of Environmental DNA Analysis

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環境 DNA メタバーコーディングは、採捕を伴わずに魚類群集を高感度に把握できるが、様々な段階で生じるコンタミネーションが問題である。Illumina ではシーケンス時にインデックスホッピングが生じることが知られており、インデックスの誤帰属は非生息地域での偽陽性につながる。Nanopore のシーケンサーではシーケンス時に PCR を行わないため、原理的にインデックスホッピングは生じないと予想されたが、予備解析で誤帰属が認められた。そこで本研究では、魚類 eDNA 解析の PCR およびライブラリ調製条件を段階的に検討し、誤帰属の発生要因を突き止め、低減法を確立した。

さらに Nanopore のシーケンスデータは 95% 程度であるが、コンセンサス配列を取得し変異解析を行うことで、精度 99.9% のシーケンスデータを取得し、一塩基違いの配列でも区別可能なパイプラインを開発した。

Environmental DNA (eDNA) metabarcoding enables highly sensitive characterization of fish communities without the need to capture organisms. However, contamination introduced at various stages of the workflow remains a major concern. In Illumina sequencing, index hopping is known to occur during sequencing, and index misassignment can lead to false-positive detections in areas where the target species is not present. Because Nanopore sequencing does not involve PCR amplification during the sequencing process, index hopping was theoretically expected not to occur. Nevertheless, barcode misassignment was observed in our preliminary analyses.

In this study, we systematically evaluated PCR and library-preparation conditions for fish eDNA analysis, identified the factors responsible for barcode misassignment, and established a method to minimize its occurrence.

Although raw Nanopore sequencing reads typically have an accuracy of approximately 95%, we developed a bioinformatics pipeline that achieves 99.9% sequence accuracy by generating consensus sequences and performing variant analysis. This pipeline enables the discrimination of sequences differing by as little as a single nucleotide.

【P-105】

サテライト領域は環境 DNA の新たなマーカーとなりえるか？

Can satellite regions be a novel marker for environmental DNA?

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核内のサテライト領域は高い反復性を持つため環境 DNA 分析の有望なマーカー候補であると考えられるが、現在は環境 DNA 分析の検出系は主にミトコンドリア DNA や核内の ITS 領域といった限られたゲノム領域で検討されており、その有用性は未知である。本研究ではサテライト DNA を対象として、環境 DNA 分析に有効な検出系の設計が可能であるのか検証した。チュウゴクスッポン (*Pelodiscus sinensis*) の全ゲノム配列から高コピー数で存在するサテライト DNA を抽出し、その領域内に検出系を設計した。他の淡水ガメも含めた組織 DNA によるリアルタイム PCR によって本検出系の特異性が確認され、さらに本種の生息が既知である河川で採水された環境サンプルにおいても検出が確認された。このことから、サテライト領域が環境 DNA 分析の有効なマーカーであることが実証された。

Nuclear satellite regions are considered a promising candidate marker for eDNA assays due to their highly repetitive. However, eDNA assays are mainly designed against a limited set of genomic regions, most notably mitochondrial DNA and the nuclear internal transcribed spacer region, and the usefulness of satellite regions remains unexplored. In this study, we tested the feasibility of designing an effective eDNA assay by targeting satellite DNA in the nuclear genome. Using the whole-genome sequence of the Chinese softshell turtle (*Pelodiscus sinensis*) we identified a region of highly repetitive satellite DNA and designed a new assay. Real-time PCR using tissue-derived DNA from the target species and other locally occurring freshwater turtles confirmed the specificity of the assay, and the target species was successfully detected in environmental water samples collected from a river in which it is known to live. Thus, our study demonstrated that satellite DNA can be a novel marker for eDNA assay.

【P-106】

ハイブリダイゼーション MIG-seq (hyMIG-seq) 法による環境 DNA からの標的種の選択的解析

Selective analysis of target species from environmental DNA using hybridization capture-based MIG-seq (hyMIG-seq)

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ターゲットキャプチャー法は、標的 DNA 配列に相補的なビオチン修飾プローブをハイブリダイズさせ、磁気ビーズによって標的 DNA 断片を選択的に濃縮する手法である。本研究では、環境試料から特定種の DNA 情報を効率よく取得することを目的として、この手法とゲノムワイド SNP 分析法である MIG-seq 法を組み合わせた hyMIG-seq 法を開発した。ブナおよびスダジイの葉から抽出した DNA を用いてプローブを作製し、森林土壌から抽出した環境 DNA を分析した。手法の有効性を検証するため、①試料中の標的種 DNA 量を変化させた場合の検出感度の評価、②通常の MIG-seq 法との比較、③異なる樹種由来プローブを用いた特異性評価を行った。その結果、本手法により環境試料から特定種のゲノムワイド SNP を選択的に濃縮・検出できることが確認された。さらに実用性を評価するため、河川水から抽出した環境 DNA への適用を進めている。

The target capture method uses biotin-labeled probes complementary to target sequences and streptavidin-coated magnetic beads to selectively enrich target DNA fragments from extracted DNA samples. In this study, we combined this technique with MIG-seq, a genome-wide SNP genotyping method, and developed a hybridization capture-based MIG-seq (hyMIG-seq) approach to obtain genome-wide SNP information for specific species from environmental samples. We prepared target-specific probes from DNA extracted from leaves of two tree species, *Fagus crenata* and *Castanopsis sieboldii*. Using these probes, we analyzed environmental DNA extracted from forest soil. To evaluate the performance of the method, we conducted three experiments: (1) evaluating detection sensitivity by varying the amount of DNA from the target species in environmental samples, (2) comparing hyMIG-seq with conventional MIG-seq, and (3) assessing specificity using probes derived from different species. The results demonstrated that hyMIG-seq can effectively detect and enrich genome-wide SNPs of target species from environmental samples. The method is currently being applied to environmental DNA extracted from river water to further evaluate its practical applicability.

【P-107】

昆虫汎用 primer をめざして — MtlInsects-16S の改良 —

Improvement and Enhanced Detection Performance of Universal Insect eDNA Metabarcoding Primers (MtlInsects-16S) Targeting Mitochondrial 16S rRNA

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昆虫類を対象とした環境 DNA 解析に関して、mtDNA 16S rRNA 領域内に短鎖 DNA（約 200 bp）を高効率で増幅する汎用プライマー「MtlInsects-16S」を開発し、高い検出性能を実証した。しかし、トビケラ目やハエ目など約 1 割の分類群において、プライマー配列のミスマッチを確認し、検出効率が低下する可能性が明らかとなった。そこで本研究では、これらの不一致を補う改良プライマーを再設計し、1st PCR 条件の最適化を行った。検証の結果、従来の MtlInsects-16S を用いた結果と比較して改プライマーの検出種数や精度について議論し、昆虫類における網羅的生物多様性評価、生物モニタリングへの有用性を議論する。

For environmental DNA analysis of insects, we previously developed “MtlInsects-16S”, a universal primer set targeting a short (~200 bp) region within the mtDNA 16S rRNA region, which demonstrated high detection performance. However, sequence mismatches were identified in approximately 10% of taxa, including Trichoptera and Diptera, raising concerns about reduced detection efficiency. In this study, we redesigned complementary primers to address these mismatches and optimized the 1st PCR conditions. Validation against the original MtlInsects-16S set demonstrated that the modified primers successfully increased the number of detected species and improved analytical resolution. In this presentation, we compare the detection performance between the original and modified primer sets and discuss the utility of the improved MtlInsects-16S system for comprehensive biodiversity assessment and biological monitoring of insect communities.

【P-108】

個体レベル環境 DNA 分析への展開を目指したフローサイトメトリーによる環境水中細胞・核の選択的分取

A flow cytometry-based cell isolation approach toward individual-level eDNA analysis

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現行の環境 DNA 分析は、多数個体由来の DNA 混合物を環境からまとめて抽出するため、個体識別や交雑判定など、個体レベルの解析を行うことは困難である。そこで本研究は、フローサイトメトリー技術を応用し、環境水中の高等動物由来細胞・核を選択的に分取してシングルセル解析へ展開する手法を検討した。ミナミメダカをモデルとし、環境水からの細胞・核濃縮法を検討するとともに、細胞・核を識別する染色条件を設定した。その後、セルソーターを用い、前方散乱光および蛍光強度に基づき細胞・核と推定される集団を分取した。分取画分を蛍光顕微鏡観察およびリアルタイム PCR により解析したところ、細胞・核が高純度に分取されていることが確認された。さらに簡易マイクロ流路デバイスを用いることにより、分取画分からシングルセル（核）単離が可能であることも確認された。本分析アプローチは、将来的な個体レベル環境 DNA 分析への展開が期待される。

Current environmental DNA (eDNA) analysis is based on the extraction of DNA assemblages originating from multiple individuals, which hinders the recovery of individual-level genetic information, such as individual identification and hybrid detection. In this study, we explored a novel approach that integrates flow cytometry into eDNA analysis by selectively isolating animal cells and nuclei from environmental water. Using Japanese medaka (*Oryzias latipes*) as a model, we first optimized methods for concentrating cells and nuclei from environmental water and established staining conditions for their identification. Cell sorting was then performed based on forward scatter and fluorescence intensity to isolate a putative cell/nucleus population. The sorted fraction was evaluated by fluorescence microscopy and real-time PCR, confirming the successful isolation of a highly enriched cell/nucleus fraction. Furthermore, we demonstrated that individual cells/nuclei could be isolated from the sorted fraction using a simple microfluidic device. This analytical approach has the potential to pave the way for future individual-level eDNA analysis.

【P-109】

PCR 産物はどのように広がるのか？ —キャリーオーバーコンタミネーションの拡散メカニズムと対策—

How do PCR products spread ? —Mechanisms of carryover contamination and prevention strategies

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環境 DNA 分析では微量 DNA を PCR により増幅して検出するため、PCR 産物によるコンタミネーション（コンタミ）は偽陽性の主要因となり、その対策は大きな課題である。しかし、PCR 産物によるコンタミの発生機序については十分に解明されていない。筆者はノロウイルスおよびコロナウイルス検出用 PCR キットを開発する中で、陰性判定を基本とする高感度検査系におけるコンタミ問題に長年取り組んできた。その過程で、環境中の PCR 産物を検出する評価系を構築し汚染分布を調査した。その結果、従来考えられていたエアロゾルによる空間拡散ではなく、ドアノブ、ピペット、遠心機操作部など作業者が接触する箇所から高頻度に PCR 産物が検出され、手指を介した接触伝播が汚染経路であることを明らかにした。本発表では、PCR 検査系で得られた知見を基にコンタミを防止するための実験操作上の留意点、および効果的な除染方法について紹介する。

Environmental DNA (eDNA) analysis relies on PCR amplification to detect trace amounts of DNA; therefore, contamination by PCR products is a major cause of false-positive results and remains a critical challenge. However, the mechanisms underlying PCR product contamination are not fully understood. Through the development of PCR-based detection kits for norovirus and coronavirus, we have investigated contamination issues in highly sensitive assays where negative results are essential. In this study, we established a detection system for environmental PCR products and analyzed their distribution in laboratory environments. Contrary to the conventional assumption that aerosol-mediated dispersion is the primary contamination route, PCR products were frequently detected on surfaces contacted by operators, including door handles, pipettes, and centrifuge controls. These findings demonstrate that hand- and glove-mediated contact transmission is a major pathway for PCR product contamination. We present practical guidelines for preventing contamination during PCR workflows and effective decontamination methods based on these findings.

【P-110】

長鎖 eDNA の収量向上に向けたろ過後フィルター保存法の開発

Development of a post-filtration filter preservation method for increasing the yield of long-fragment eDNA

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長鎖 eDNA の利用は、より正確な種判別や詳細な種内多型の評価を可能にする。しかし、従来の eDNA 分析は主に短鎖を対象としており、長鎖の適切な回収・保存法に関する知見はほとんどない。そこで本研究では、長鎖 eDNA の収量向上を目的に、ろ過後フィルターの保存に適した試薬および保存温度を検討した。まず、琵琶湖の水をろ過したフィルターをエタノール、Buffer-ATL、RNA-later および BAC にそれぞれ浸漬して 4 °C で 1 週間保存し、バス類の Cyt *b* 領域、1500、2000 および 2500 bp の DNA 断片の増幅産物濃度を比較した。さらに、エタノールおよび ATL では、4 °C と -20 °C での保存効果を比較した。その結果、エタノールおよび ATL に浸漬して -20 °C で保存した場合、非処理より有意に高い収量が得られることが示唆された。本結果は、野外における長鎖 eDNA の利用拡大に寄与すると期待される。

The use of long-fragment environmental DNA (eDNA) enables more accurate species identification and detailed assessment of intraspecific genetic variation. However, conventional eDNA analysis has primarily focused on short fragments, and there is little knowledge regarding appropriate methods for recovering and preserving long-fragment eDNA. In this study, we examined appropriate preservation reagents and storage temperatures for post-filtration filters to improve the recovery of long-fragment eDNA. First, water samples collected from Lake Biwa were filtered, and the filters were individually immersed in ethanol, Buffer-ATL, RNA-later or BAC and stored at 4 °C for one week. Then, we compared the concentrations of PCR amplicons for DNA fragments of 1,500, 2,000, and 2,500 bp, targeting the Cyt *b* region of *Micropterus* sp. In addition, for filters preserved in ethanol or Buffer-ATL, we also compared the effect of storage at 4 °C and -20 °C. The results suggested that filters immersed in either ethanol or Buffer-ATL and stored at -20 °C yielded significantly higher DNA concentrations compared to control samples. These findings are expected to contribute to expanding the applicability of long-fragment eDNA in the field.

【P-111】

ダム湖における魚類環境 DNA の水平的不均一性と採水地点設定への示唆

Horizontal heterogeneity of fish eDNA in a reservoir and its implications for sampling site selection

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ダム湖における環境 DNA (eDNA) の水平分布を把握するため、ダム湖の左岸から右岸にかけて採水を行い、魚類を対象とした eDNA メタバーコーディング解析を実施した。調査は 2019 年に福島県阿武隈川水系の三春ダムで行った。採水は、湖岸部では岸沿いに 5m 間隔で 5 地点、沖部では湖岸から約 30m 間隔を基本とした 11 地点で実施し、表層水 1L を採取した。分析の結果、湖岸部では近接する地点間における検出魚種の差は小さく、採水位置の違いによる影響は小さいと考えられた。一方で、左右岸では検出魚種の一部に差がみられ、湖岸環境の違いにより検出しやすさが異なる種の存在が示唆された。沖部では流入河川由来の eDNA を反映した可能性のある魚種が比較的多く確認された。本発表では、追加の採水・分析結果を含め、ダム湖における環境 DNA の水平分布の一事例を示すとともに、その結果を踏まえた採水地点設定上の留意点について報告する。

To investigate the horizontal distribution of eDNA in a dam reservoir, fish eDNA metabarcoding was conducted using water samples collected along a transect extending from the left bank to the right bank of the dam reservoir. The study was carried out in 2019 at Miharu Dam, located in the Abukuma River system, Fukushima Prefecture, Japan. Sampling was conducted at five shoreline sites spaced 5 m apart and eleven offshore sites established at approximately 30 m intervals from the shoreline. One liter of surface water was collected at each site. The results showed that differences in detected fish species among neighboring shoreline sites were generally small, suggesting that sampling location had a limited influence on species detection at this spatial scale. In contrast, some differences in detected species were observed between the left and right shorelines, indicating that shoreline environmental conditions may affect the detectability of certain species. In offshore areas, relatively large numbers of species potentially reflecting eDNA transported from inflowing rivers were detected. In this presentation, we will report additional sampling and analytical results and present a case study of horizontal eDNA distribution in a dam reservoir. In this presentation, we will present a case study of horizontal eDNA distribution in a dam reservoir, including additional sampling and analytical results, and summarize key considerations for sampling-site selection based on these findings.

【P-112】

沿岸域における魚類環境 DNA 濃度のばらつきの階層間比較およびその濃度・環境依存性

Hierarchical comparison of variability in fish environmental DNA concentrations in coastal ecosystems and their concentration and environmental dependencies

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環境 DNA 分析における定量精度を高める上で、濃度のばらつきがどのように生み出されるのかを理解することは重要である。本研究では、舞鶴湾沿岸域で採水を行い、マアジ (*Trachurus japonicus*) 環境 DNA 濃度のばらつきの階層間比較およびその濃度・環境依存性を調べた。ばらつきに対する各階層の相対的な寄与度は濃度帯によって異なり、全データではばらつきの 90% 以上が時点・地点レベルに起因したのに対して、低濃度帯データでは qPCR レベルの寄与度は 70% にまで増加した。また、環境パラメータは、qPCR 繰り返し間での濃度の変動係数 (CV) と部分的に関連していた一方、サンプル間での CV とは関連しなかった。特に限られた予算・リソースの下で環境 DNA の定量精度を効果的に高めるためには、予想される濃度、対象種の密度、環境条件に合わせて、サンプル数や繰り返し数が柔軟に調整される必要がある。

Understanding variability in environmental DNA (eDNA) concentration is essential for improving the precision of quantitative eDNA analyses. In this study, seawater samples were collected from Maizuru Bay to investigate the variability in Japanese jack mackerel (*Trachurus japonicus*) eDNA concentrations across hierarchical levels (sampling time points and sites, samples, and qPCR replicates) and their concentration- and environmental dependencies. The relative contribution of each level to the variation differed depending on the concentration range; whilst more than 90 % of the total variance in eDNA concentration in the overall data was explained by differences among sampling sites and time points, the relative contribution of the qPCR level to the total variance increased up to 70 % under low-concentration conditions. Additionally, some environmental parameters were correlated with the coefficient of variation (CV) of eDNA concentration at the qPCR level, whereas they were not correlated with the CV at the sample level. To effectively improve the precision of eDNA quantification, particularly within a limited budget and resources, optimal sampling and replication strategies should be flexibly tailored to expected eDNA concentrations, organismal densities, and environmental conditions.



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