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要旨集 Abstracts

【P-01】

環境 DNA を用いたサンショウウオ類の季節動態と定量性の評価

Seasonal dynamics and quantitative evaluation of salamanders using environmental DNA

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環境 DNA(eDNA) は両生類の分布や動態把握に有効であり、特に幼生期の種判別が困難で成体の採捕も稀なサンショウウオ類に有用と考えられる。本研究では、サンショウウオを対象にメタバーコーディングによる定量性評価と季節動態の把握を目的とした。福島県猪苗代町の池と河川で定期採水と捕獲調査を行い、相対リード数と個体数の対応を検証した。その結果、止水環境ではクロサンショウウオ（*Hynobius nigrescens*）およびトウホクサンショウウオ（*H. lichenatus*）の相対リード数が幼生や卵数と相関し、季節変動を良好に反映した。一方、流水環境ではバンダイハコネサンショウウオ（*Onychodactylus intermedius*）が周年確認され定量性はさらなる検討が必要であった。以上より、eDNA はサンショウウオ類のモニタリングに有効であり、調査設計には生息環境や採水時期の考慮が重要である。

Environmental DNA (eDNA) is effective for assessing the distribution and dynamics of amphibians and is particularly useful for salamanders, whose species identification during the larval stage is difficult and whose adult capture is rare. In this study, we aimed to evaluate the quantitative performance of eDNA metabarcoding for salamander monitoring and to assess their seasonal dynamics. Regular water sampling and capture surveys were conducted in a pond and a stream in Inawashiro, Fukushima Prefecture, and the relationship between relative read counts and individual numbers was examined. In the pond environment, relative read counts of *Hynobius nigrescens* and *H. lichenatus* correlated with the number of larvae and eggs, reflecting seasonal variation effectively. In contrast, *Onychodactylus intermedius* was observed throughout the year in the stream, but its quantitative relationship with eDNA requires further investigation. These results demonstrate that eDNA metabarcoding provides a reliable approach for monitoring salamanders, with effective study design requiring careful consideration of habitat characteristics and the timing of water sampling.

【P-02】

環境 RNA に着目した絶滅危惧種オキサンショウウオの幼生・亜成体特異的プライマーの検証

Evaluation of stage specific primers (larvae and subadults) for the endangered salamander *Hynobius okiensis* using environmental RNA

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島根県隠岐諸島の固有種であるオキサンショウウオは絶滅危惧種に指定されている。しかし、本種の生態の多くが未知であるため、効果的な保全が困難である。そこで本研究では、野外の環境水に含まれる mRNA（環境 RNA）を検出することで、本種の幼生や成体などの成長段階ごとの生息状況や季節性を明らかにし、保全活動の一助にできるのではと考えた。そこでまず、幼生と亜成体の体組織サンプルを用いた RNA-seq 解析によって各成長段階で特異的に発現する mRNA を同定し、環境 RNA 検出用のプライマーを作製した。つぎに、これらプライマーを用いたリアルタイム PCR を実施して、本種の飼育水から各成長段階に特異的な mRNA を検出できるかどうかを検証した。その結果、SYBR Green 法では成長段階特異的な mRNA の検出はできなかった。現在、Taqman Probe 法を用いたプライマーの再検証を行う準備を進めている。

The Oki Islands endemic (*Hynobius okiensis*) is classified as an endangered salamander species. Due to a lack of ecological information on this species, particularly at the adult stage, effective conservation remains challenging. The purpose of this study was to clarify the habitat status and seasonal occurrence of each growth stage, including larvae and adults, by detecting environmental mRNA (eRNA) in water sample. First, RNA-seq analysis of tissue samples from larvae and subadults identified mRNAs specifically expressed at each growth stage, and primers for mRNA detection were designed. Next, using these primers, we conducted real-time PCR to test whether stage-specific eRNA could be detected in incubation water containing individuals at each growth stage. As a result, stage-specific eRNA could not be detected using the SYBR Green assay. We are currently preparing to validate the primers again through TaqMan probe assays.

【P-03】
環境 DNA 分析によるオオサンショウウオの遺伝的多様性の解明

Elucidation of the genetic diversity of the Japanese giant salamander through eDNA analysis

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オオサンショウウオ（*Andrias japonicus*）は日本固有種で世界最大級の両生類であり、岐阜県以西の西日本に広く分布する。しかし、生息環境の変化やチュウゴクオオサンショウウオとの競争・交雑などにより生息数が減少している。そのため本種は絶滅危惧 II 類及び国の特別天然記念物に指定されており、サンプル入手が困難である。また夜行性かつ日中は水中の岩場に隠れて生活するという生態ゆえに、従来の直接の観察や捕獲による調査は時間と労力を要する。そこで本研究は、環境 DNA 分析を用いることで、非侵襲的かつ効率的にオオサンショウウオの遺伝的多様性を解明することを目的とした。先行研究で得られた、オオサンショウウオの分布域を幅広く網羅した河川サンプルを用いてサンガーシーケンス解析した。その結果、環境 DNA 分析により河川ごとに配列情報を得て、遺伝的多様性を解明できる可能性が示唆された。

The Japanese giant salamander (*Andrias japonicus*) is an endemic species to Japan and one of the world's largest amphibians, widely distributed in western Japan, west of Gifu Prefecture. However, its population has declined due to changes in habitat and competition/hybridization with the Chinese giant salamander. Consequently, this species is designated as Vulnerable (Category II) and is also a National Special Natural Monument, making sample acquisition difficult. Furthermore, its nocturnal habits and daytime concealment among underwater rocks make traditional direct observation and capture surveys time-consuming and labor-intensive. Therefore, this study aimed to non-invasively and efficiently elucidate the genetic diversity of the Japanese Giant Salamander using eDNA analysis. Using river samples obtained from previous studies that broadly covered the giant salamander's distribution range, Sanger sequencing analysis was performed. The results suggest that environmental DNA analysis holds the potential to obtain sequence information for each river and elucidate genetic diversity.

【P-04】
環境水と魚体表の菌叢比較

Comparison of microbial communities in environmental water and fish body surfaces

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本研究では、魚体表粘液の菌叢と、生育する環境水の菌叢の関連を明らかにすることを目的に、市販淡水魚を異なる 2 か所の環境水間で 4 回移動させ、魚体表粘液および環境水の菌叢の経時変化を、16S rRNA を標的としたメタバーコーディングアプローチにより解析した。主座標分析結果から、4 回の移動時すべてで、魚の体表菌叢のプロットは、日数の経過とともに各環境水の菌叢のプロットに近づく傾向を示した。生育環境を移動することで、魚体表の菌叢の多様性も生育環境の多様性に合わせて変化する可能性が示された。

This study aimed to clarify the relationship between the microbial communities in fish body surface mucus and those in the environmental water where the fish grow. To achieve this, commercially available freshwater fish were transferred four times between two different environmental water sites. The temporal changes in the microbial communities of both the fish body surface mucus and the environmental water were analyzed using a metabarcoding approach targeting 16S rRNA.Principal coordinate analysis results showed that, during all four transfers, the plots of the fish surface microbiota tended to approach the plots of the respective environmental water microbiota as days passed. This suggests that transferring the rearing environment may cause the diversity of the fish surface microbiota to change in response to the diversity of the new rearing environment.

【P-05】
魚類感染症罹患時における病原体および宿主由来環境核酸量の推移

Changes in environmental nucleic acid levels from pathogens and hosts associated with fish infection

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環境 DNA（eDNA）を用いた魚類の生息量推定には、様々な要因の影響を考慮する必要があり、その一つに魚類の疾病がある。アユなどでは河川環境下で感染症がしばしば発生し、大量死に至ることもある。特に、体表の潰瘍や出血を伴う疾病は、宿主からの eDNA 放出量に影響を及ぼす可能性が高い。しかし、感染症罹患時における宿主魚類の eDNA 放出動態に関する情報は乏しい。本研究では、アユのビブリオ病およびヤマメの伝染性造血器壊死症（IHN）を対象に感染実験を行い、飼育水中における病原体および宿主由来核酸濃度をデジタル PCR により定量した。その結果、両病原体とも感染後に宿主由来の eDNA 濃度は増加傾向を示した。一方で、疾病によって病原体と宿主の eDNA 放出動態が異なる可能性が示唆された。本研究は、eDNA を用いた魚類生息量推定技術の精度向上に加え、魚類疾病対策への応用にも貢献することが期待される。

Environmental DNA (eDNA) is a powerful tool for estimating fish biomass; however, its accuracy can be influenced by various factors, including disease outbreaks in fish populations. In river environments, infectious diseases affecting species such as ayu (*Plecoglossus altivelis*) are prevalent and can cause mass mortalities. These diseases, often accompanied by skin ulcers and hemorrhaging, may substantially affect the amount of eDNA released by the host. Nevertheless, little is known about how eDNA release dynamics change during the progression of disease.In this study, we evaluated the changes in the concentration of eDNA in the rearing water of ayu and yamame (*Oncorhynchus masou masou*) experimentally infected with vibriosis, a bacterial disease, and infectious hematopoietic necrosis (IHN), a viral disease, respectively. Water samples were collected before the infection and periodically thereafter to quantify pathogen-derived and host-derived nucleic acids using digital PCR.Our results showed that host-derived eDNA concentrations increased considerably following infection by both pathogens. However, the timing of increases in pathogen- and host-derived eDNA varies depending on the disease. These findings suggest that disease and disease type influence eDNA release dynamics, underscoring the potential of eDNA analysis not only for estimating fish biomass but also as a tool for monitoring and managing fish diseases.

【P-06】

積雪期・融雪出水期の森林渓流における魚類環境 DNA の検出特性

Detection characteristics of fish environmental DNA in forest streams from the snow season to the snowmelt flooding period

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本研究では、積雪寒冷地の河川において最も典型的・周期的な出水攪乱である「融雪出水」に着目し、森林渓流で積雪期から出水期、渇水期にかけた環境 DNA 調査を行うことで、環境 DNA 調査の出水攪乱に対する生物の応答を明らかにするポテンシャルについて考察した。

北海道に位置し、約 9 割が森林に覆われた単一流域内の 9 ～ 10 地点において、2 月中旬（積雪期）、4 月中旬（出水ピーク）、5 月下旬（出水後）、7 月中旬（渇水期）の 4 季にわたって環境 DNA サンプルを収集し、標準 DNA を用いた定量的な MiFish 法に供試した。その結果、いずれの季節も地点ごとに類似の群集構造が捉えられたものの、流量と採水量で補正したコピー数は 2 月のみ極端に少ないなど、検出特性に月ごとの違いがみられた。環境 DNA 調査は出水に対する魚類の応答を調べるのに有用と考えられるが、積雪期の環境 DNA 検出量を低下させる要因など更なる検討の余地もある。

Snowmelt flooding is the most typical disturbance on streams in snowy regions. In order to discuss the potential of environmental DNA (eDNA) surveys for revealing the responses of organism to flood disturbance, we conducted multiple eDNA sampling in a forest mountain stream from the snow season to the drought period after snowmelt flooding.

We collected eDNA samples from the same 9–10 sites within a single watershed in Hokkaido, where approximately 90% of the catchment area is covered with forest, over four seasons: mid-February (snow covered), mid-April (flood peak), late May (flood almost ending), and mid-July (low water). Samples were subjected to the quantitative MiFish analysis with three standard DNA. Detected fish communities were similar across seasons, but eDNA copies (per [sampled volume / flow rate]) varied seasonally. Especially, samples collected in February showed particularly lower values in all sites. While eDNA surveys have potential to reveal fish responses to flooding, further investigation is needed to identify factors reducing eDNA discharge (or detectability) during snow season.

【P-07】

Evaluating the feasibility of eDNA metabarcoding for detecting overwintering fish in ice-covered environments

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Investigating the species diversity and behavior of fish that overwinter under ice cover is a promising application of eDNA techniques. However, its feasibility remains underexplored. To facilitate the use of eDNA in ice-covered waters, we conducted on-ice sampling in Saroma-ko Lagoon, Japan, and Cambridge Bay, Canada. Using eDNA metabarcoding, we successfully detected a total of 40 and 29 fish taxa from under-ice water in the lagoon (including its inlet river) and the bay, respectively. Our results demonstrate that the composition of fish eDNA under ice is both spatially and temporally heterogeneous. In Saroma-ko Lagoon, species composition estimated from eDNA apparently differed between under-ice seawater and river water. Short-term temporal changes were also observed within a lagoon site. In Cambridge Bay, a site near an offshore island showed greater taxonomic variability across the sampling period (about one month) and between sampling depths (2 m and 10 m) compared to a nearshore site. These heterogeneities in eDNA distribution likely reflect site-specific differences in under-ice fish communities and the temporal turnover of species composition. Furthermore, we detected fish eDNA in sea ice meltwater, suggesting that particulate matter containing fish eDNA is incorporated during ice formation, although the specific process is not yet clear. Our findings confirm the detectability of fish eDNA in ice-covered environments and highlight the importance of understanding eDNA dynamics in frozen environments to improve the validity and applicability of this technique.

【P-08】
国内外来魚類オヤニラミの LAMP 法を用いた迅速な環境 DNA 分析法の開発

Development of LAMP-based environmental DNA detection method of the domestic invasive fish *Coreoperca kawamebari*

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外来種は生物多様性を劣化させ生態系の不可逆的に改変するため、効果的な管理が必要である。特に分布拡大の対策が重要で、早期発見と防除が必須である。近年、国内外来種として分布を拡大しているオヤニラミ *Coreoperca kawamebari* は在来生態系に影響を及ぼす危険性の高い淡水魚類である。外来種の生息状況や分布を評価するために環境 DNA 分析が利用されるが、既存の PCR 法は機材や試薬の制約より現場でのモニタリングに高コストを要する。本研究では LAMP 法による等温反応を用いて、環境 DNA からオヤニラミを特異的に検出できるプライマーセットを開発した。オヤニラミ、コウライオヤニラミ *C. herzi* の組織由来の DNA サンプルに適用し特異性を確認した結果、オヤニラミ由来のサンプルのみで白色沈殿を伴う陽性反応が得られた。この技術は、安価で迅速な環境 DNA 分析であり、外来種管理への貢献が期待される。

Invasive species degrade biodiversity and cause irreversible ecosystem changes, requiring effective management strategies. Among these, preventing range expansion through early detection and rapid response is critical. The freshwater fish *Coreoperca kawamebari*, which has recently expanded as a domestic invasive species in Japan, poses a high risk to native ecosystems. Environmental DNA (eDNA) analysis has become an increasingly useful tool for evaluating the presence and distribution of such invasive species. However, conventional PCR-based methods often require costly equipment and reagents, limiting their feasibility for on-site monitoring. To address this issue, we developed a primer set for isothermal amplification using loop-mediated isothermal amplification (LAMP) that enables the specific detection of *C. kawamebari* from eDNA samples. Specificity tests were conducted using tissue-derived DNA from *C. kawamebari* and the closely related *C. herzi*. Results demonstrated that positive reactions, confirmed by white precipitate formation, occurred only in samples derived from *C. kawamebari*, indicating clear species-specificity of the assay. This LAMP-based detection system provides a rapid and low-cost alternative to conventional PCR approaches. The method offers practical advantages for field-based eDNA monitoring and is expected to contribute significantly to invasive species management through more efficient detection and distribution assessment of target taxa.

【P-09】

Disentangling seasonality and uncertainty in riverine fish detections through seasonal eDNA surveys

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Environmental DNA (eDNA) surveys have become widely used for seasonal biodiversity monitoring due to their efficiency and simplicity in replicated field sampling. However, detection patterns often vary even within the same season because of the complexity of natural ecosystems. Here, we analyzed fish eDNA metabarcoding data from the 17-km Isazu River system in northern Kyoto, collected over two years of seasonal surveys, to distinguish species with consistent versus inconsistent seasonal detection patterns between two years and to identify those exhibiting uncertain detections under given conditions. Between May 2023 and February 2025, we collected 96 eDNA samples from 12 sites along the main channel and its tributary, spanning from downstream to upstream reaches. Species detections obtained through MiFish metabarcoding were compared across identical seasons using the weighted Jaccard similarity index, which accounted for detections at the same or adjacent sites. The mean Jaccard index across 49 species was 0.543 (SD = 0.390). Similarity was lowest in summer (mean = 0.363, SD = 0.358), and 22 species showed a similarity of zero due to non-detection in one of the years. Overall, species with higher detection frequencies exhibited more consistent patterns across two years. In particular, those detected in roughly one-quarter of all samples generally showed high similarity (>0.60) between years. By contrast, species with fewer detections—including diadromous fishes and brackish species—also displayed high Jaccard similarity, suggesting that eDNA surveys effectively captured their seasonality. Comparisons among downstream, midstream, and upstream habitats indicated that species inhabiting lower reaches showed more stable seasonal detections, whereas variability increased toward upper reaches. Taken together, these findings suggest that summer flow fluctuations strongly affect the midstream and upstream reaches of the Isazu River system, likely explaining both the greater variability in detection similarity there and the overall reduction in similarity observed in summer.

【P-10】

Environmental DNA dynamics during early development and physiological changes in salmon

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Environmental DNA (eDNA) has become an powerful tool for ecological monitoring, yet its relationship with organismal development remains poorly understood. Previous studies suggested that reproductive behavior and developmental stages can influence eDNA concentrations, but little is known about changes before and after key developmental events such as hatching. To address this gap, we investigated eDNA dynamics during the early ontogeny of chum salmon (*Oncorhynchus keta*), focusing on physiological and behavioral transitions from eggs to fry. Using controlled rearing experiments, we quantified eDNA flux across three developmental stages: egg, alevin, and fry. Our results revealed striking stage-specific differences. Immediately after hatching, eDNA flux rose sharply and then stabilized throughout the alevin stage. A dramatic increase occurred at the transition to the fry stage, with flux approximately 30-fold higher than in the alevin stage (linear mixed model and post hoc Tukey–HSD test: p0.05). These findings demonstrate that eDNA production is tightly linked to developmental progression in salmon. In particular, the transition from alevin to fry represents a critical threshold where eDNA release intensifies substantially, likely reflecting enhanced metabolism and activity. Our study provides novel evidence that eDNA signals can serve as indicators of developmental stages, offering new opportunities for assessing hidden life stages such as those occurring under gravel in salmonid spawning habitats.

【P-11】

eDNA 分析感度におけるマーカー依存的ボトルネックとその克服：ウナギ特異的レトロトランスポゾンによる改善を例に

Marker-dependent bottleneck in eDNA sensitivity: A breakthrough with eel-specific retrotransposons

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eDNA 分析は、生物分布を調べる効率的な手法だ。しかし、従来法は殆どの場合ミトコンドリア DNA を標的としており、サンプル中のマーカー数が検出感度におけるボトルネックであった。特に、重要な水産資源であると同時に絶滅危惧種でもあるニホンウナギの調査では、感度の向上が求められている。本研究はこの課題に対応すべく、ウナギ（Anguilla 属）のゲノムに豊富に存在するレトロトランスポゾン「UnaSINE1」を標的とする検出系を開発し、従来のミトコンドリアマーカーと性能を比較した。河川調査で SINE マーカーはミトコンドリアマーカーの平均 170 倍以上のコピー数を示した。またミトコンドリアマーカーでは 81 検体中 32 検体でウナギ属 DNA が検出されたのに対し、SINE マーカーでは 62 検体で検出された。分類群特異的な反復配列の活用は、サンプリングや試薬コストを増やさず感度を大幅に向上させる実用的方法である。

Environmental DNA (eDNA) analysis is an efficient tool for investigating the distribution of organisms. However, in most cases, mitochondrial DNA is targeted, and the limited number of such markers in samples has been a potential bottleneck for detection sensitivity. In particular, for the Japanese eel (Anguilla japonica), which is both a commercially important and endangered species, more sensitive detection methods are required for reliable field monitoring. To address this, we developed a novel eDNA assay targeting UnaSINE1, a retrotransposon abundant in the genome of Anguilla eels, and compared its performance with a standard mitochondrial marker. In river surveys, the UnaSINE1 marker exhibited averaging more than 170-fold higher copy numbers than the mitochondrial marker. Additionally, UnaSINE1 was detected in 62 out of 81 samples compared to 32 for the mitochondrial marker. Using such taxon-specific repetitive sequences is a practical and cost-effective way to greatly improve eDNA detection sensitivity.

【P-12】
ニホンウナギの環境 DNA 濃度の日周変化と活動量との関係

Relationship between diel variation in Japanese eel (*Anguilla japonica*) eDNA concentration and activity

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環境 DNA 濃度は対象種の生物量の指標となる一方で、水温などの環境条件や対象種の活動に影響されることが示唆されている。したがって、適切なモニタリングには、これらの影響を野外で明らかにする必要がある。本研究ではニホンウナギ *Anguilla japonica* を対象とし、2024 年 11 月と 2025 年 5 月に、2 時間間隔で 24 時間の採水を行い、環境 DNA 濃度の日周変化を調べた。それと同時に PIT タグを用いて個体行動を追跡した。その結果、活発に活動しない 11 月には昼夜間の環境 DNA 濃度に差が無いのに対して、活動が活発化する 5 月には夜間に環境 DNA 濃度が高くなった。さらに、採水前 1 時間における PIT タグ装着個体の検出個体数が増加すると、環境 DNA 濃度も増加する傾向がみられた。これらの結果は、本種の夜行性という特性を反映しており、夜間の活動個体数の増加が、環境 DNA 濃度に影響を与えることが示唆された

Environmental DNA (eDNA) concentration is increasingly used as an indicator of target species biomass. However, it is also influenced by environmental factors (e.g., water temperature) and the activity of the target species. Understanding the extent these influences affect eDNA concentrations under field conditions is essential for reliable monitoring. In this study, we investigated diel variation in eDNA concentration of the Japanese eel *Anguilla japonica*. On two occasions (November 2024 and May 2025), water samples were collected every two hours over a 24-h period. Simultaneously, individual movements of Passive Integrated Transponder (PIT) tags were tracked. In November, when eel activity is generally less intense, eDNA concentrations did not differ between day and night. By contrast, in May, when eel activity generally increases, eDNA concentrations were consistently higher at night. Moreover, eDNA concentrations tended to increase with the number of PIT-tagged individuals detected during the hour before sampling. These findings indicate that the diel variation in eDNA concentration reflects the nocturnal behavior of Japanese eels, with increased nighttime activity elevating eDNA levels. Our study demonstrates that eDNA concentrations are shaped not only by biomass but also by the target species' behavioral rhythms. This highlights the importance of considering diel activity patterns when designing eDNA monitoring protocols for nocturnal fish like *A. japonica*.

【P-13】
熱ストレス下におけるニジマスの組織特異的環境 RNA 放出の評価

Assessment of tissue-specific environmental RNA release in rainbow trout under heat stress

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環境 DNA 濃度は個体の生理状態を反映すると考えられるが、単一の指標では要因解析が困難である。環境核酸は主に皮膚・鰓・消化管などの上皮組織に由来し、熱などの環境ストレスに対して異なる影響を受けることが知られている。本研究ではニジマスの組織特異的 mRNA を標的としたマーカーを開発し、水槽実験で熱ストレス応答の指標となるかを検証した。稚魚をストレス群と対照群で飼育し、経時的 eRNA を採取し、それぞれ皮膚、鰓、消化管に由来する keratin 5、ZG16、cdhr2 を対象に RT-qPCR で解析した。その結果、熱ストレス群では皮膚および鰓由来の eRNA 濃度が高く、とくに鰓に特異的な ZG16 は有意に増加した。一方、消化管に由来する cdhr2 には明確な差はみられなかった。これらの知見から、eRNA を活用した非侵襲的な熱ストレス評価手法の有用性や、環境応答に伴う環境核酸の動態について議論する。

Environmental DNA concentration has been considered to reflect the physiological state of organisms, but analyzing causal factors based on this single indicator remains difficult. Environmental nucleic acids are thought to originate mainly from epithelial tissues such as skin, gills, and intestines, which are known to be differently affected by environmental stressors including heat. In this study, we developed multiple tissue-specific mRNA markers in rainbow trout (Oncorhynchus mykiss) and tested whether changes in their composition could be used to monitor stress responses. Juveniles were reared under heat-stressed and control conditions in tanks, and water samples were collected over time. eRNA was extracted and quantified by RT-qPCR targeting keratin 5 (skin), ZG16 (gill), and cdhr2 (intestine), with 28S rRNA used for normalization. The heat-stressed group showed higher concentrations of skin- and gill-derived eRNA, and gill-specific ZG16 increased significantly. In contrast, intestine-derived cdhr2 showed no clear difference. These tank experiments suggest that thermal stress increases the release of transcripts from specific tissues, which can then be detected as eRNA in surrounding water. We discuss the potential of this approach as a non-invasive method for assessing thermal stress in fish and the implications for understanding the release dynamics of environmental nucleic acids under stress conditions.

【P-14】

2021 年から 2025 年にかけての宮城県南三陸町志津川湾における魚類群衆の変化

Changes in the fish fauna in Shizugawa Bay, Minamisanriku Town, Miyagi prefecture from 2021 to 2025

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志津川湾は、寒流と暖流の影響を強く受ける。冬期は水温が低下し、暖水性種の越冬は稀だった。2023 年以降、湾内で高水温が継続したことで暖水性種が多く観察され、魚類群衆構造が大きく変化したと予想される。そこで、2021 年 5 月から 2025 年 1 月にかけて志津川湾の沿岸で採水された環境 DNA データを用いて、群集構造の年変化と環境要因の関係性を検討した。検出された総 OTU 数は 1215 で、同定の結果 406 の科、属もしくは種に分類できた。dbRDA を行ったところ、2024、2025 年と 2021、2022 年のサンプルが採水年の効果によって分かれ、2023 年のサンプルはどちらにも含まれた。このことから、2023 年に発生した黒潮の極端な北編が、その前後の魚類相の差に表れたと考えられる。同定された一部の分類群では、北方性種から南方性種への置き換わりが観測されていることから、今後も追跡調査が重要である。

Shizugawa Bay is an area where cold and warm currents alternate seasonally. In this area, water temperatures decreased in winter, with warm-water species rarely surviving the season. Since 2023, however, persistently high-water temperatures in the bay are expected to have increased warm-water species and significantly altered fish community composition. Therefore, we examined the relationship between annual changes in community composition and environmental factors using all environmental DNA data collected along the coast of Shizugawa Bay from May 2021 to January 2025. A total of 1,215 OTUs were detected and classified into 406 families, genera or species following identification. Distance-based redundancy analysis (dbRDA) revealed that the 2024–2025 and 2021–2022 samples separated due to the sampling year effect, while the 2023 samples were included in both clusters. This suggested that the extreme northward shift of the Kuroshio Current in 2023 showed the difference in fish communities before and after the event. As water temperatures decreased below 9° C in winter 2025, it is likely that warm-water species did not survive the winter. In some identified taxa, a shift from cold-water species to warm-water species has been observed, and it is important to continue monitoring these changes.Therefore, follow-up surveys are important in the future.

【P-15】
環境 DNA を用いた氾濫原プール魚類群集評価の基礎的検討

Basic study for fish assemblage evaluation in floodplain pools using environmental DNA

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本研究では，環境 DNA 分析を用いて河道内氾濫原に存在する氾濫原プールの魚類群集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，2024 年の 10 月（出水前）と 11 月（出水後）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，統計解析（単回帰分析・多変量解析）を用いて各月における環境 DNA 分析と採捕調査による魚類群集データの関係を検討した．結果として，多くの魚種の環境 DNA 濃度と採捕による魚類個体数との間に出水前・出水後ともに顕著な正の相関関係がみられ，環境 DNA 濃度が出水前後の氾濫原プールにおける魚類の相対的な生物量を反映していることが示された．

In this study, we examined if the fish assemblage evaluation in floodplain pools is possible using environmental DNA (eDNA) analysis. 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 October (pre-flood month) and November (post-flood month) 2024. We calculated the eDNA concentrations of each fish via filtration, extraction, and quantitative eDNA metabarcoding. Then, we examined fish assemblage concordances between the eDNA data and electrofishing data, using univariate and multivariate statistics. As a result, the eDNA concentration of most fishes were significantly and positively correlated with fish populations from the electrofishing on both months, indicating that it is possible to evaluate the fish assemblage structure in the floodplain pools before and after the flood event using eDNA.

【P-16】

環境 DNA 定量メタバーコーディングを用いた徳島県吉野川下流域における魚類群集の多様性パターンについて

Quantitative environmental DNA metabarcoding reveals fish biodiversity patterns in the lower reaches of Yoshinogawa River basin in Tokushima

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本研究では、徳島県吉野川流域下流部における魚類多様性のパターンを、環境 DNA 定量メタバーコーディング手法を用いて解析しました。旧吉野川本流、流入支川、農業用水路の計 90 地点で採水を行い、各地点の魚類群集のα多様性を比較しました。さらに、冠水深が異なる板野地区（浅い）および鳴門地区（深い）におけるβ多様性パターンも評価しました。各地点で検出された魚種数は 5 ～ 35 種でした。α多様性は旧吉野川本流で最も高く、次いで支川、農業用水路の順でした。この結果は、旧吉野川本流の多様な生息環境が多様な魚類を支えていることを示唆しています。非計量多次元尺度法（NMDS）解析の結果、板野地区と鳴門地区では魚類群集構成に違いが認められ、局所的な魚類群集構成が冠水深の影響を受けている可能性が示されました。

In this study, we investigated fish diversity patterns in the downstream region of the Yoshino River basin, Tokushima Prefecture, using quantitative eDNA metabarcoding. Water samples were collected from 90 sites, including the main channel of the Kyu-Yoshinogawa River, its tributaries, and agricultural waterways. We compared the α-diversity of fish assemblages among these habitats. Furthermore, we evaluated β-diversity patterns between areas with different inundation depths: Itano (shallow) and Naruto (deep).The number of fish species detected per site ranged from 5 to 35.The highest α-diversity was observed in the main channel of the Kyu-Yoshinogawa River, followed by its tributaries and agricultural waterways. This finding suggests that the diverse habitats within the main channel support a wide variety of fish species. Non-metric multidimensional scaling (NMDS) analysis revealed distinct differences in fish assemblage composition between the Itano and Naruto areas, indicating that local fish assemblages may be influenced by inundation depth.

【P-17】

eDNA metabarcoding of St. Lawrence River basin in Quebec, Canada

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MiFish universal primers have been widely used to investigate global fish distributions. In this study, we applied MiFish metabarcoding to assess fish communities in the St. Lawrence River basin, Quebec, Canada. The St. Lawrence River originates from Lake Ontario and flows approximately 1,200km to the Gulf of St. Lawrence and the Atlantic Ocean. Our river sampling sites were located in the river's midsection, between Montreal and Quebec City. We also collected water samples from nearby lakes, including those in the Eastern Townships and in Temiscouata regions. MiFish analysis of the river mainstem detected 47 molecular operational taxonomic units (MOTUs), including two sturgeon species native to the region (Atlantic and Lake sturgeon). Analyses of lakes in the Eastern Townships and Temiscouata detected 21 and 18 MOTUs, respectively. In the Eastern Townships lakes, the MOTUs were dominated by yellow perch, rock bass, and bluegill, whereas in Temiscouata lakes, they were dominated by creek chub and other dace species. Notably, only five MOTUs were shared between the two lake regions, indicating distinct fish communities not only between the river and lakes but also among lakes in different regions. We also applied MiMammal primers, which detected MOTUs from Muscrat, North American beaver, and Eastern gray squirrel among others. While caution is warranted due to limited sample sizes and uncertainties in DNA reference sequences, our results highlight the power and broad applicability of MiFish and MiMammal primers for biodiversity assessment worldwide.

【P-18】

Comparing eDNA metabarcoding and open-access observation data for freshwater biodiversity in northern Taiwan

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Assessing biodiversity is fundamental for understanding ecosystem processes and biogeography, as well as for supporting conservation and resource management. Traditionally, biodiversity assessments have relied on field observations and specimen records, approaches that are often labour-intensive and spatially uneven. In recent years, environmental DNA (eDNA) metabarcoding has emerged as a powerful molecular tool capable of detecting a wide range of taxa from water samples with high sensitivity. This has raised the question of whether eDNA metabarcoding could replace traditional, visual-based survey methods in biodiversity assessments. In this study, we compared eDNA metabarcoding results with open-access observation data to evaluate freshwater biodiversity across northern Taiwan. Water samples were collected from 22 riverine sites and analysed by targeting a 420 bp fragment of the mitochondrial cytochrome b (cytB) gene for vertebrate taxa. Detected sequences were taxonomically assigned using QIIME2 and MIDORI Reference 2 database and compared with species occurrence records obtained from TaiBIF, Taiwan's node of the Global Biodiversity Information Facility (GBIF). Preliminary analyses show that while TaiBIF records contained a broader overall spectrum of taxa, eDNA data revealed clearer fine-scale spatial variation in biodiversity and detected cryptic components of freshwater communities. TaiBIF data also exhibited strong spatial and temporal biases, with minimal information available for several surveyed rivers. Taken together, our findings demonstrate that eDNA metabarcoding and open-access observation databases are complementary. Integrating both approaches can enhance freshwater biodiversity monitoring, inform conservation strategies, and improve our understanding of biogeographic patterns in Taiwan and beyond.

【P-19】
三陸沿岸における魚類群集の変遷

Transition of fish community in Sanriku coast

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沿岸諸地域は、気候変動に伴う海洋環境の変化に晒されており、水産業などへの影響が懸念されている。三陸沖では、2023 年から黒潮の北方移入により、平年差から 6 度も上昇する、世界的に類を見ない大規模かつ広域的な水温上昇を経験している。この海洋環境変動が生物群集へ及ぼす影響を明らかにすることは、水産業の盛んな三陸の沿岸生態系の保全のために重要である。

本研究では、2022 年から年 3 回、三陸沿岸（宮古〜女川）にある 18 の湾、各 3 地点で魚類を対象とした環境 DNA メタバーコーディング調査を行い、地点ごとにおける群集組成の時間的変動を追跡することで、水温上昇が群集組成に与える影響を明らかにする。予備的な解析では、種構成に対する緯度勾配の影響を評価したが、緯度勾配のみでは十分説明できない。これは、各地域の局所的な環境によるものかもしれない。本発表では湾の特性やそれに付随する水質の影響を含めて議論する。

Coastal regions are exposed to changes in the marine environment due to climate change, raising concerns about impacts on fisheries and related industries. Off Sanriku, since 2023, the shift in Kuroshio Current path has caused an drastic and wide ranging rise in sea temperature which rarely observed worldwide. Clarifying how such marine environmental changes affect biological communities is essential for the conservation of coastal ecosystems in Sanriku, where fisheries play a vital role. In this study, we have been conducting environmental DNA metabarcoding surveys targeting fish since 2022, three times a year, at three sites within each of 18 bays along the Sanriku coast (from Miyako to Onagawa). By tracking temporal changes in community composition at each site, we aim to reveal the effects of rising sea temperature on fish communities. Preliminary analyses evaluated the influence of the latitudinal gradient on species composition, but this factor alone does not sufficiently explain the observed patterns. This may be due to local environmental conditions in each bay. In this presentation, we will further discuss the influence of bay-specific characteristics and associated water quality on community composition.

【P-20】

Contrasting deep-sea and coastal surface fish communities: An environmental DNA metabarcoding study of vertical stratification in Suruga Bay

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Environmental DNA offers a powerful, non-invasive method for assessing marine biodiversity across different ecological zones. There is an urgent need to explore deep sea regions of which we know very little about because of the high costs involved in any attempt to observe that environment. This study compares two distinct fish communities in Suruga Bay, Japan, using eDNA metabarcoding: one from available data from samples collected at a 400m intake in Yaizu[1], and another from a coastal sea surface sample in Mihonomatsubara.

The analysis revealed a stark contrast between the two sites,, confirming strong vertical stratification of fish communities. The Mihonomatsubara surface sample was dominated by coastal species like Girella punctata and Mugil cephalus. In contrast, the 400m Yaizu sample was characterized by mesopelagic families, including Myctophidae (lanternfish), which were identified as key indicator species for the deep-water environment. Despite the profound differences, four species were common to both datasets. The presence of the vertical migrator Benthosema pterotum highlights direct ecological linkage between the zones. Furthermore, the detection of the freshwater species Cyprinus carpio in both samples underscores the influence of riverine outflow on both surface and deep-sea ecosystems or some other factor.

Understanding and demonstrating the use of eDNA metabarcoding as an effective tool for characterizing and differentiating vertically structured marine ecosystems will help in future expansion and establishing a baseline for future deep sea monitoring, effects of climate change and human intervention on deep sea species.

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【P-21】

Long-term monitoring of fish communities using eDNA metabarcoding

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Monitoring of fish communities is crucial for assessing the health of marine ecosystems. Environmental DNA (eDNA) metabarcoding is an emerging technique for detecting genetic DNA from marine organisms in water samples. Recent advances in mitochondrial region amplification, high-throughput DNA sequencing, and taxonomic annotation have made eDNA metabarcoding a rapid and non-invasive tool for studying fish communities. However, long-term monitoring of temporal and spatial variation using eDNA metabarcoding remains little studied. To address this gap, we conducted monthly sampling of marine water from Wanghaixiang Chaojing Bay Resource Conservation Area (WCBRCA), Dapin coast and Changtanli fishing port in the North of Taiwan from 2023 to 2024. Results indicate the detection of a total of 570 species, with 267, 348, and 347 species in WCBRCA, Dapin coast, and the fishing port, respectively. Among the species detected in over 50% of samples, most in WCBRCA and Dapin coast were reef-associated and demersal species, whereas half of the fish assemblages in the fishing port were pelagic. These findings reveal shifts in eDNA signals over distances of several kilometers, providing evidence that eDNA monitoring can improve our understanding of the spatial and temporal heterogeneity of fish communities.

【P-22】
水産有用種と同様の分布変化を気候変動に対して示す魚種集団の探索

Exploring the fish assemblages shifting their distribution alongside commercially important fishes in response to climate change

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水産有用種の資源解析に使用される漁獲データからは、個体群パラメータは得やすい一方、水温・塩分等の生息環境の網羅的把握は困難であった。そこで環境DNA情報を用いて様々な魚種の生息環境特性を把握し、気候変動に対して同様に分布を変化させる魚種集団を探索する解析を実施した。日本全国の魚類の環境DNAデータを用い、日本における資源評価対象種および検出頻度が高い魚種に対し、分布水温と塩分の範囲を推定した。この分布水温・塩分に基づき、同じ分布環境に生息する魚種グループへの区分を行った。また、同所的に出現することが多い魚種をまとめた魚種コミュニティを探索し、区分した。そしてこれらの両区分が合致した集団を「気候変動に対して同様に分布を変化させる魚種集団」と考えた。結果として、マダイはスジハゼやハオコゼといった種と同じ分布変化を示すなど、有用種と同じ分布変化をする魚種の組み合わせが数十種単位で明らかとなった。

Stock assessment of commercially important fishes is generally conducted using catch data because it can provide valuable population parameters. However, it has been difficult to comprehensively understand habitat characteristics, such as the ranges of habitat water temperatures and salinities for target species. Therefore, we aimed to develop a new method utilizing environmental DNA metabarcoding to understand environmental characteristics of various species and to explore groups shifting their distribution similarly with climate change. The ranges of habitat water temperatures and salinities were estimated for the species subject to stock assessment in Japan, as well as for the species frequently etcted in the eDNA data. Based on these environmental parameters, the fish species were classified into several groups. In addition, most species were categorized into several fish communities based on their sympatric occurrences. We propose that assemblages composed of species belonging to the same environmental groups and fish communities represent “fish assemblages shifting their distribution alongside commercially important fishes in response to climate change”. As a result, we identified dozens of combinations of commercially important fish species and other species that are shifting their distribution in tandem. For example, the distribution shift of *Pagrus major* (Japanese red seabream) were found to be similar to that of *Acentrogobius virgatulus* and *Paracentropogon rubripinnis*.

【P-23】

海洋魚類群集における市民科学者と職業科学者の環境 DNA データ比較と活用可能性

Comparison and potential use of environmental DNA data between citizen professional scientists and scientists in marine fish communities

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近年、市民科学者は生物多様性モニタリングの新たな担い手として注目されているが、そのデータの研究利用における信頼性は十分に検証されていない。本研究では、日本全国沿岸で行われた市民ボランティアによる環境 DNA 調査の信頼性を検証するため、①調査状況に関する報告内容、②得られたデータの生物群集構造に関して科学者による調査と差異があるか検討した。トピックモデルを用いた分析では、調査状況の両者の報告に明確な違いが認められた。これは、市民ボランティアの報告には研究者が重視する内容が欠落している可能性を示唆する。一方、dbRDA による分析では、主要魚種の組成など群集構造にほとんど差がみられなかった。以上の結果より、市民ボランティアによる環境 DNA 調査は、生物の群集構造を分析する上で科学者と同等の信頼性を有する一方で、調査状況の報告には改善の余地が残ることが明らかになった。

In recent years, citizen scientists have gained attention as new contributors to biodiversity monitoring, yet the reliability of their data for research use has not been sufficiently examined. In this study, we evaluated the reliability of environmental DNA (eDNA) surveys conducted by citizen volunteers along the Japanese coast by comparing them with surveys conducted by professional scientists, focusing on (1) the contents of survey reports and (2) differences in community structure derived from the collected data. Topic modeling revealed clear differences in survey reporting between the two groups, suggesting that citizen volunteers may omit information typically emphasized by researchers. In contrast, distance-based redundancy analysis (dbRDA) indicated little difference in major fish species composition or overall community structure. These findings suggest that eDNA surveys conducted by citizen volunteers can provide community structure data with reliability comparable to that of scientists, while also highlighting the need to improve the reporting of survey conditions.

【P-24】

Fish fauna beneath the coastal sea ice in Lützow-Holm Bay, East Antarctica, revealed by environmental DNA

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The coastal sea ice around the Antarctic continent provides the basis of the ecosystem for a wide range of organisms, although its extent is highly sensitive to climate change. Monitoring under-ice biota is essential for understanding rapidly changing coastal ecosystems of the Southern Ocean. We investigated the fish fauna beneath the coastal sea ice in Lützow-Holm Bay, East Antarctica - an area where surveys have been limited because of the thick ice and the resulting inaccessibility - using environmental DNA (eDNA) metabarcoding.

A total of 14 fish species were detected, including four not previously reported from this region. Among the detected species, nine belonged to the nototheniid fishes (Nototheniidae, Perciformes), which dominate the Southern Ocean. Relative abundances of Amplicon Sequence Variants (ASVs) were particularly high for the benthic emerald rockcod *Trematomus bernacchii* (over 60%) and the cryobenthic (i.e., dwelling on the ice underside) bald notothen *Pagothenia borchgrevinki* (over 30%). The predominance of bald notothen is likely attributable to the extensive year-round sea ice cover over most of the bay's coastal areas. Near Adélie penguin *Pygoscelis adeliae* breeding colonies, fish-derived eDNA concentrations were consistently low. In particular, eDNA from the bald notothen was almost absent in the vicinity, suggesting the scarcity of bald notothen, likely due to penguins' predation pressure. On the other hand, approximately 1.9% of ASVs could not be assigned to species level. This study demonstrates the utility of eDNA metabarcoding for surveying Antarctic fish fauna, while highlighting the need to expand reference databases and develop primers tailored for Antarctic fishes.

【P-25】

Environmental DNA metabarcoding for the monitoring and assessment of marine biodiversity and network interactions in coastal waters

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Effective monitoring of marine biodiversity is critical for ecological conservation and the preservation of fragile ecosystems. Traditional methodologies, which rely predominantly on morphological identification and field expertise, are often invasive and disruptive, posing substantial risks to sensitive environments such as coral reefs. Addressing these limitations, eDNA metabarcoding has emerged as a non-invasive and highly effective approach for assessing marine biodiversity and evaluating the impacts of anthropogenic activities. In this study, we present our recent application of eDNA metabarcoding to monitor and evaluate marine biodiversity through both active and passive sampling techniques. Unlike conventional methods, which frequently fail to detect rare species and inadequately capture the full taxonomic diversity, eDNA metabarcoding—augmented by the use of multiple marker genes—enables a comprehensive examination of marine communities across diverse domains and trophic levels. This approach provides a powerful tool for investigating potential species interactions and deciphering the intricate dynamics of marine community co-occurrence networks in coastal ecosystems. We report initial findings from the application of eDNA metabarcoding with multiple marker genes to assess potential community network interactions in the coastal waters of Hong Kong. The biodiversity assessments derived from eDNA metabarcoding were cross-validated and compared with results from traditional visual surveys. This case study demonstrates the transformative potential of eDNA metabarcoding as a cornerstone for non-invasive, next-generation marine monitoring, positioning it as a vital complement to existing biodiversity monitoring programs and advancing the field of marine ecological research.

【P-26】
環境 DNA メタバーコーディングを活用した沖ノ鳥島周辺海域の生物相調査

Biodiversity survey in waters around the Okinotorishima Islands using environmental DNA metabarcoding

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国境離島周辺の広大な排他的経済水域における海底資源利用が注目されているが，本土から離れた海域では生物多様性の把握や環境モニタリングが困難である。そのため，環境 DNA 分析が有用と示唆されるが，深海域での事例は限定的であり，AUV による画像調査との比較研究もほとんど行われていない。本研究では，日本最南端の沖ノ鳥島周辺海域において，環境 DNA 分析と AUV による生物相調査を実施し，さらに過去の採捕調査結果とも比較することで，その有効性を検討した。その結果，環境 DNA は短期間に多数の生物種を確認できた。さらに本調査では駿河湾深部で近年新種記載されたヨコヅナイワシを検出でき，環境 DNA 分析が調査困難な深海魚類の分布や生態解明に有効な手法であることが改めて示された。一方で採水タイミングによる検出種数の相違，画像解析による生物相調査結果との相違，深海生物のデータベース登録の問題などの課題が示された。

In recent years, the utilization of seafloor resources within the vast Exclusive Economic Zones (EEZs) around remote islands has attracted increasing attention. However, biodiversity assessment and environmental monitoring in such offshore areas remain challenging. Environmental DNA (eDNA) analysis has emerged as a promising approach, yet applications in deep-sea environments are still limited, and comparative studies with Autonomous Underwater Vehicle (AUV) imagery are rare.In this study, we conducted both eDNA analysis and AUV-based benthic fauna surveys around the Okinotorishima Islands, the southernmost point of Japan, and further compared the findings with past capture-based data. Our results show that eDNA enabled the rapid detection of numerous species, underscoring its efficiency for deep-sea biodiversity assessment. Notably, we detected the Yokozuna Slickhead (*Narcetes shonanmaruae*), a species recently described from Suruga Bay, for the first time outside its previously known range. This new distribution record demonstrates the effectiveness of eDNA analysis for revealing the distribution and ecology of elusive deep-sea fishes that are otherwise difficult to study. On the other hand, several challenges were highlighted, including differences in detected species depending on sampling timing, discrepancies between eDNA and AUV survey results, and limitations related to incomplete deep-sea reference databases.

【P-27】

Marine community genetics from environmental DNA: A test in the Hawaiian Islands

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Environmental DNA (eDNA) has the potential to provide a more comprehensive perspective on community-wide population genetics by increasing species sampled in meta-analyses to hundreds. However, a key challenge for using eDNA is determining haplotype frequencies from mixed environmental samples containing many individuals and taxa. We investigated if eDNA accurately described mtDNA diversity and genetic differentiation for 18 marine reef fish and mammals previously studied with individual, tissue-based methods. eDNA successfully recovered dominant haplotypes at frequencies consistent with previous individual-based approaches. Furthermore, measures of sequence diversity were significantly correlated between approaches. In contrast, population structure metrics were only weakly correlated, as eDNA often detected stronger and more frequent genetic differentiation than tissue-based methods when read counts were used as proxies for individuals. Overall, eDNA successfully converted complex mixed samples into informative proxies for haplotype frequencies, enabling accurate characterization of genetic diversity even at low sample sizes, providing a pathway toward community-wide population genetic analyses.

【P-28】
島根県指定希少野生動物ミナミアカヒレタビラの生息実態解明に向けた環境 DNA 調査

Environmental DNA survey to clarify the habitat status of the endangered southern red tabira bitterling (*Acheilognathus tabira jordani*) in Shimane Prefecture

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ミナミアカヒレタビラは山陰や北陸の限られた地域に生息する絶滅危惧種である。島根県においては本種の生息地がいくつか確認されているが、各個体群の減少は著しく、保全に向けた生息実態の把握は急務である。そこで発表者らは、本種の環境 DNA 検出系（盛山ら 2024）を用いて、県内の河川 2 地域で環境 DNA 調査を実施し、本種の分布状況や季節移動性の解明を進めている。その結果、本種の生息が確認されていた地点においては概ね環境 DNA が検出されている一方で、非検出の地点も確認されており、個体群の更なる減少が懸念される。現在、同所的に生息する侵略的外来種タイリクバラタナゴの種特異的な環境 DNA 検出系の開発にも着手している。今後、これら 2 種の環境 DNA 調査を実施し、生息地の重複などを明らかにして、ミナミアカヒレタビラの優先的に保全すべき場所の特定などを行う予定である。

The southern red tabira bitterling (*Acheilognathus tabira jordani*) is an endangered species found only in limited areas in the San'in and Hokuriku regions of Japan. Using an eDNA detection system (Moriyama et al. 2024), we conducted surveys in two river catchments in Shimane Prefecture to evaluate this species' habitat distribution and seasonal migratory patterns. As a result, eDNA of the species was often detected at sites where its presence had been confirmed. However, in several other sites, no eDNA was detected, suggesting a decline in local populations. We have also developed a species-specific eDNA detection system for the invasive rosy bitterling (*Rhodeus ocellatus ocellatus*). In the future, by using both eDNA detection systems, we will clarify the habitat overlaps between the two species and identify priority sites for the conservation of *A. tabira jordani*.

【P-29】
大阪湾におけるキジハタの分布および産卵場所の推定

Estimation of the distribution and spawning grounds of *Epinephelus akaara* in Osaka Bay, Japan

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キジハタ *Epinephelus akaara* は高い市場価値を有する一方、乱獲等により資源量が減少し、絶滅危惧ⅠB類に指定されている。本種の分布特性と繁殖動態の把握を目的に、非侵襲的かつ効率的な環境 DNA 分析を適用した。本研究では特に核 eDNA とミトコンドリア eDNA の比率に着目し、産卵行動の推定を試みた。調査は 2 年間にわたり実施し、初年度は大阪湾 23 地点において 7 ～ 11 月に月 1 回の採水を行い、nu-DNA および mt-DNA を標的とした検出系により解析した。次年度は調査地点を 15 に絞り、月 1 回の定期採水に加えて夜間採水を行い、日内変動の把握を試みた。その結果、藻場や消波ブロックといった沿岸環境が分布要因の一つであることが示され、さらにこれらの環境が繁殖地としても寄与する可能性が示唆された。これらの知見は、本種の生態的特徴の理解を深めるとともに、資源保全や持続的利用に向けた基盤情報を提供する。

The red-spotted grouper *Epinephelus akaara* is a high-value marine resource species whose population has declined due to overfishing, leading to its designation as endangered. To understand its distributional and reproductive dynamics, we applied eDNA analysis, which is a non-invasive and efficient monitoring approach. This study focused on the ratio of nuclear to mitochondrial eDNA (nu-eDNA/mt-eDNA) as a potential indicator of spawning activity. Field surveys were conducted over two years in Osaka Bay, Japan. In the first year, water samples were collected monthly from July to November at 23 sites, and analyses were performed using assays targeting the nuclear ITS1 region and the mitochondrial cytb region. In the second year, surveys were conducted at 15 selected sites with both monthly sampling and additional nighttime sampling to examine diel variation in eDNA concentrations. The results demonstrated that coastal habitats with seagrass beds and wave-dissipating blocks are important factors influencing the distribution of *E. akaara*. These habitats may also contribute to the occurrence of reproductive behavior, as indicated by spatiotemporal changes in the nu-eDNA/mt-eDNA ratio. These findings not only advance our ecological understanding of this endangered species but also provide fundamental knowledge for its conservation and sustainable management. The integration of eDNA approaches, particularly those incorporating nuclear-to-mitochondrial ratios, represents a promising tool for detecting spawning activity and identifying critical habitats, thereby supporting effective strategies for the recovery of *E. akaara* populations.

【P-30】
環境 DNA を用いた北日本沿岸におけるアイナメ属分布推定

Coastal distribution of *Hexagrammos* in northern japan inferred from environmental DNA

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生物の分布を把握することは生態学的理解や環境変動の評価において重要である。近年、日本近海では海水温上昇が予測され、特に北日本において顕著とされる。アイナメ属（*Hexagrammos*）は、この海域に分布境界をもち環境変動の指標種となり得る。そこで、本研究では日本に生息するアイナメ属全種であるアイナメ（*H. otakii*）、クジメ（*H. agrammus*）、ウサギアイナメ（*H. lagocephalus*）、エゾアイナメ（*H. stelleri*）、スジアイナメ（*H. octogrammus*）を識別可能なユニバーサルプライマーを設計した。組織および環境水由来の DNA 解析の結果、MiFish ではアイナメ属単一 OTU として検出されていた 4 種それぞれの識別に成功した。さらに、北日本の複数地点において本プライマーを適用して環境 DNA 解析を行ったので、その結果を報告する。

Understanding species distributions is fundamental to ecological inference. Around Japan, sea surface temperature is predicted to rise, especially in northern regions. *Hexagrammos* species have a distributional boundary in these regions. Accordingly, shifts in species-level presence at these areas may serve as practical indicators of climate-related range dynamics. Traditional capture-based surveys require substantial effort and may miss rare species, whereas environmental DNA surveys help address these limitations. We designed a primer set capable of identifying all five *Hexagrammos* species occurring in Japan: *H. otakii*, *H. agrammus*, *H. lagocephalus*, *H. stelleri*, and *H. octogrammus*. Analyses of DNA from tissue and environmental water samples showed that the newly developed primer set has successfully identified all five species. Our eDNA metabarcoding analyses detected at least one operational taxonomic unit (OTU) of *Hexagrammos* in all five water samples, comprising three OTUs from Hokkaido and three OTUs from Honshu. Among these, an OTU of *H. octogrammus* was shared between the two islands, whereas OTUs of *H. lagocephalus* and *H. stelleri* were found exclusively in Hokkaido. These results demonstrate the suitability of this method for characterizing distributional differences in *Hexagrammos* and for identifying environmental factors that shape these patterns.

【P-31】

水生生物の胃内容物および鳥類の排泄物を対象とした種特異的 DNA 検出系によるホシガレイ *Verasper variegatus* 種苗の捕食者の特定

Identifying predators of hatchery-reared juveniles of spotted halibut *Verasper variegatus* by species-specific DNA detection from stomach contents of fishes and crustaceans and bird feces

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栽培漁業では、放流後における人工種苗の生残・分散の把握が放流効果の向上を目指す上で重要である。福島県松川浦では商業的価値が高いホシガレイ *Verasper variegatus* の種苗が継続的に放流されている。先行研究では、複数の捕食者による被食が種苗個体の減耗の要因とされたが、目視観察の困難さから捕食者の特定はできていない。本研究は DNA 分析手法により捕食者を特定するため、2023 ～ 2025 年に松川浦湾口の放流区画において、放流後の夜間に採集した魚類・甲殻類の胃内容物、および鳥類営巣地より採取した排泄物を用いて定量 PCR によるホシガレイ DNA の検出を試みた。その結果、全ての分類群からホシガレイ DNA を種特異的に検出した。したがって、本研究で用いた手法は、多様な捕食者によるホシガレイ種苗の被食減耗の評価において有効なツールと言える。

In stock enhancement programs, understanding the post-release dispersal and survival of hatchery-reared juveniles is crucial for improving the effectiveness of stocking. In Matsukawa-ura Lagoon, Fukushima Prefecture, juveniles of spotted halibut *Verasper variegatus* have been continually released to enhance the stock. Previous studies suggested that one of the major causes of post-release mortality is predation by multiple piscivorous species, but direct evidence identifying predators has been lacking due to the difficulty of visual observation. This study used DNA-based techniques on samples from 2023 to 2025 to identify predators. To detect spotted halibut DNA, stomach contents of fish and crustaceans collected at night after the release, as well as fecal samples from bird nesting sites, were analyzed using species-specific quantitative PCR. Spotted halibut DNA was detected from all taxonomic groups examined, indicating predation by a variety of predators. These results demonstrate that this approach provides an effective tool for evaluating predation-related mortality of the released juveniles.

【P-32】

環境 DNA を用いた慣行田んぼと谷津田における無脊椎動物の生物群集の季節変化比較

Comparison of seasonal changes in invertebrate communities in conventional and valley rice paddies using environmental DNA

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茨城県で隣接する慣行田んぼと谷津田で昆虫類を対象にした環境 DNA 調査を実施し、非特異増幅などの分析上の課題及び水生昆虫類の季節変化が把握可能性を検討した。使用したプライマーは MtInsects-16S プライマーであり、流水環境では高い種検出率を誇るプライマーである。

調査は慣行田んぼが水を張っている 6 月から 8 月に実施したが、緩流部ではミジンコ由来の非特異増幅が若干確認されたものの、全体として大きな分析上の問題は確認されず、多くの止水性昆虫を検出することが可能であった。季節変化では 6 月の調査ではいずれの田んぼでも止水性昆虫の種数が同程度であったのに対して、8 月になるにつれ慣行田んぼの種数が減少する傾向がみられた。これは過去の捕獲による調査結果と同じ傾向であり、環境 DNA 調査が田んぼの生物相を的確に把握可能であることを示していると考えられた。

An environmental DNA survey for insects was carried out in adjacent conventional rice paddies and valley rice paddies in Ibaraki Prefecture to investigate analytical issues such as non-specific amplification and the possibility of understanding seasonal changes in aquatic insects. The primer used was the MtInsects-16S primer, which has a high species detection rate in running water environments.

Surveys were conducted from June to August, when the conventional rice fields were filled with water. Although some non-specific amplification from Daphnia magna was observed in slow-flowing areas, overall no major analytical problems were identified and it was possible to detect many aquatic insects in lentic environment. In terms of seasonal changes, while the number of species of water stopping insects was similar in all rice fields in the June survey, there was a trend towards a decrease in the number of species in the conventional rice fields through the seasons. This was the same trend as in previous capture surveys, and was considered to indicate that environmental DNA surveys can accurately identify the biota of rice fields.

【P-33】

中国地方一級水系における環境 DNA 分析を用いた水生昆虫類の多様性調査に関する基礎的検討

Basic study for assessing aquatic insect diversity using an environmental DNA analysis in first grade rivers in Chugoku district

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生物の生息・分布情報は、生態系の健全性を評価する上で重要な情報である。特に、水生昆虫類は河川の環境変化に敏感であることから、河川生態系における指標種となる。一方で、水生昆虫類の調査には高い専門性や多大な調査努力が必要であり、より効率的な調査手法の確立が求められる。そこで本研究では、河川生態系の水生昆虫類の多様性評価における環境 DNA 分析の基礎的検討を目的として、中国地方の一級河川 5 水系（佐波川、旭川、太田川、日野川、斐伊川）を対象とした水生昆虫類の環境 DNA メタバーコーディングを行い、水生昆虫類の多様性を明らかにした。また、過去に実施された河川水辺の国勢調査の採捕調査の結果から水生昆虫の種リストを作成し、環境 DNA 分析の結果と比較した。その後、水生昆虫類の多様性調査における環境 DNA 分析の有効性および今後活用していくうえでの課題について議論した。

Biodiversity information such as distribution and richness of species is essential for assessing ecosystem health. In particular, aquatic insect is an indicator in the river ecosystem because of their sensitive response to environmental change and degradation. On the other hand, surveys for aquatic insects require high expertise and great effort, and more effective method is needed. In this study, to examine an effectiveness of environmental DNA (eDNA) analysis for assessing aquatic insect diversity in river ecosystems, we assessed aquatic insect diversity using the eDNA metabarcoding in first grade rivers in Chugoku district (Saba River, Asahi River, Ota River, Hino River, and Hii River). Additionally, we compiled the species list of aquatic insects from previous records (National census on river and dam environments) and compared with eDNA results. Subsequently, we discussed the effectiveness and challenges of eDNA analysis for aquatic insect diversity monitoring.

【P-34】

Comparing traditional and genomic biodiversity assessments of stream invertebrates across a subcontinental scale

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Environmental DNA-based biodiversity assessments show promise in generating rapid, cost-effective data that could supplement, or even replace, traditional biomonitoring methods. Here, we provide a direct comparison of aquatic invertebrate community data generated by 1) traditional morphological identification of benthic samples, 2) DNA metabarcoding of benthic samples, and 3) metabarcoding of environmental DNA collected in filtered stream water. We collected all three sample types concurrently at ~60 stream reaches in 7 watersheds distributed across the continental US. For gamma and alpha diversity, morphological approaches generated more diversity at the family and genus levels, while metabarcoding found more diversity at the species level. Compositionally, methods were similar at the family level, but showed differentiation at genus and species levels. Our work demonstrates that because genomic and traditional methods capture different levels of the invertebrate community, they can be combined to enable more effective biomonitoring and a wide range of research applications.

【P-35】

環境 DNA ハプロタイピングによる水生昆虫の個体群間交流の推定

eDNA haplotyping reveals population connectivity of aquatic insects

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Understanding the dispersal patterns and population connectivity is crucial for ecological research and conservation management. However, little is known about these processes in aquatic insects, largely because of their complex terrestrial dispersal during the adult flying stage. In this study, we developed an eDNA-based mitochondrial haplotyping approach to evaluate the genetic structure of local populations, targeting a caddisfly (*Stenopsyche marmorata*; with high dispersal ability) and mayflies (e.g., *Epeorus latifolium*; with low dispersal ability). A broad-ranging field survey conducted in the upper reaches of the Kino River system (Nara Prefecture, Japan) successfully mapped their distributions and characterized their population genetic structures, enabling the inference of dispersal patterns and population connectivity for each species.

【P-36】

北海道・東北の河川における重金属影響：水生昆虫と環境 DNA による統合評価

Integrated assessment of heavy-metal pollution in Hokkaido–Tohoku rivers using aquatic insects and eDNA

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河川における化学汚染は世界的な長期課題である．水生昆虫群集は水質変化に敏感であることから長期的な生態影響評価に用いられてきたが，一般化可能かつ適用範囲の広い応答指標は未だ十分に整備されていない．本研究では，北海道および東北地方の複数河川を対象に，各河川で水質汚染影響のないリファレンス 1 地点と影響地点 2 地点以上を設定し，水生昆虫群集の採集，環境 DNA 分析用の河川表層水採水，および水中の重金属濃度（銅，カドミウム，鉛，マンガン等）の測定を実施した．環境 DNA は COI・16S 領域を標的とする昆虫類網羅的なメタバーコーディング解析に供し，群集データを得た．その結果，短距離スケールにおいては下流への流下影響により環境 DNA による地点間分離が弱まりやすい一方，pH や金属濃度などの水質勾配が群集変化の効果サイズを説明することが示唆された．

Chemical contamination in rivers is a persistent global challenge. Aquatic insect communities respond sensitively to water quality and are widely used to assess long-term ecological impacts; however, generic, transferable response metrics remain underdeveloped. To address this gap, we surveyed multiple rivers in Hokkaido and the Tohoku region of Japan. For each river, we sampled one minimally impacted reference site and at least two impacted sites. At each site we collected benthic macroinvertebrates, surface water for environmental DNA (eDNA) analysis, and measured dissolved heavy metal concentrations (Cu, Cd, Pb, Mn, etc.). eDNA was subjected to insect-oriented metabarcoding targeting the mitochondrial COI and 16S rRNA markers to obtain community profiles. Our analyses suggest that, at short spatial scales, downstream transport can dampen eDNA-based separation among sites, whereas gradients in water chemistry—particularly pH and metal concentrations—help explain variation in the effect sizes of community change.

【P-37】

Integrating eDNA and eRNA for stream health assessment using benthic diatoms

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Environmental DNA (eDNA) and RNA (eRNA) have become powerful tools for aquatic biomonitoring, but their ecological relevance differs. eDNA can persist in biofilms and reflect accumulated environmental signals, which may under- or overestimate current stream conditions. By contrast, eRNA is short-lived and expected to provide a real-time snapshot of metabolically active communities. This study compared benthic diatom assemblages derived from eDNA and eRNA, with the goal of evaluating their potential for stream health assessment. Alpha diversity indices showed negligible differences between eDNA and eRNA, although total read abundance indicated a small effect size (Cliff's $\delta = 0.207$). Taxonomic overlap was substantial (70% shared species), but genera such as *Melosira* and *Achnanthidium* exhibited clear source-specific patterns. Community similarity analysis further revealed stronger internal consistency in eRNA-based communities within lowland streams (similarity = 0.311 vs. 0.214 for eDNA) and more pronounced between-group dissimilarities (RNA = 0.129 vs. DNA = 0.184). When applying the Trophic Diatom Index (TDI), eDNA reflected cumulative water quality conditions, whereas eRNA better captured present ecological states. These results suggest that eDNA provides broad taxonomic coverage, while eRNA resolves active ecological responses with higher sensitivity. Importantly, integrating eDNA and eRNA offers a dual perspective: long-term environmental history and real-time stream health. Our findings demonstrate the practical value of incorporating eRNA into diatom-based bioassessment frameworks to enhance the accuracy and resolution of freshwater ecosystem monitoring.

【P-38】
隠岐近海のプランクトン相の通年メタバーコーディング解析

Year-round metabarcoding analysis of plankton communities in the waters near Oki Islands

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隠岐近海は、対馬海流を沿って移動する生物群の回遊経路にあたる。我々の研究室の先行研究にて、隠岐の加茂漁港を起点として調査地点を 5 箇所設定し、9 月と 12 月にプランクトンネットと表層採水による野外調査を行なった。COI 配列を用いた網羅的なメタバーコーディングによる解析の結果、季節によって DNA が検出される棘皮動物の種には違いがみられること、野外環境中から棘皮動物の幼生プランクトンの DNA を検出できることが分かった。そこで本研究では通年のプランクトンネットサンプリングと環境 DNA メタバーコーディングを用いて、先行研究で検出された棘皮動物および頭足類を対象に隠岐近海の浮遊生物の分布状況の通年モニタリングを試みた。また、本研究では回遊している浮遊性タコ類について配列決定し、データベースの補完を行った。この結果から、環境 DNA メタバーコーディング解析による隠岐近海の頭足類の季節的消長を調査した。

Off the coast of Oki are the migration route of used by biological communities traveling with the Tsushima Warm Current. In our laboratory's previous research five research sites were selected starting from Kamo fishing port, where the Oki marine biological station is located, and moving away from the bay in stages. Field surveys were conducted in september and december using plankton nets and surface water sampling. Universal primers capable of amplifying echinoderm DNA were created to detect environmental DNA in the samples, and comprehensive analysis using metabarcoding was conducted. Metabarcoding results showed differences in the echinoderm species for which DNA was detected depending on the season. we also found that DNA of larval plankton of echinoderms could be detected from the outdoor environment by sampling with plankton nets. Quantitative PCR results showed that larval plankton DNA was detected to the same extent throughout the southern coast of the post-island area, indicating a wider migration range than initially expected. So we research to try to targeting echinoderms and cephalopods detected in previous studies and we attempted to monitor the distribution of planktonic organisms around the Oki Islands throughout the year by eDNA metabarcoding. And also this study sequenced and supplemented the database for migratory pelagic octopods. Based on these results, we investigated the seasonal fluctuations of cephalopods in the waters near Oki using environmental DNA metabarcoding analysis.

【P-39】

沖縄科学技術大学院大学サンゴプロジェクトのイシサンゴ環境ＤＮＡ調査の概要と慶良間諸島での事例

eDNA metabarcoding survey of Sclearctinian corals in the OIST cotal project and its result in the Kerama Islands

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サンゴは熱帯海洋生態系を支える最重要生物である。現在、開発や地球温暖化にともなう環境変化により、多くのサンゴ礁はダメージを受け、変化している。サンゴ礁保護のため、多くの政府・非政府組織でサンゴ礁のモニタリングが行われているものの、形態による判別の難しさゆえに、イシサンゴの分類学的に詳細な分布情報を含む調査はほとんど行われていない。OIST マリンゲノミクスユニットでは、「サンゴを知ろう、サンゴ礁を守ろう」のスローガンのもと、イシサンゴの大規模環境 DNA メタバーコーディング調査を行っている。本発表では、そのプロジェクトの概要（日本に生息するとされる 85 属のサンゴのうち、83 属を検出可能なメタバーコーディングシステムを構築したことなど）や、慶良間諸島における調査で、比較的長期（1998 年以前と現在）と短期（2023 年と 2024 年）のイシサンゴ群集の変化を記録したことを示す。

Corals are essential organisms because they support tropical marine ecosystems. Environmental changes due to anthropogenic development and global warming are currently damaging and altering many coral reefs. While many governmental and non-governmental organizations monitor coral reefs to protect them, few studies have provided detailed taxonomic information on stony coral distribution due to the difficulty of distinguishing them by morphology. The OIST Marine Genomics Unit is conducting a large-scale environmental DNA metabarcoding survey of scleractinian corals under the slogan “Learn about corals and protect coral reefs.” in the OIST coral project This presentation outlines the OIST coral project (including the development of a metabarcoding system capable of detecting 83 of the 85 genera of corals known to inhabit Japan) and presents the results of a survey in the Kerama Islands that documented long-term (before 1998 and present) and short-term (between 2023 and 2024) changes in stony coral communities by eDNA metabarcoding analysis.

【P-40】

Pristine Seas of the Pacific: Coral reef biogeography assessed using environmental DNA

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The Pacific Ocean, covering more area than all of the world's land combined, plays a central role in regulating global climate, ecological, and social systems that support humanity. In its tropical regions, coral reefs host more biodiversity than any other marine ecosystems. Large-scale studies of conspicuous fauna (primarily fishes, corals, and mollusks) have described gradients of decreasing richness moving away from the Indo-Malayan region or Coral Triangle. However, recent studies that leverage metabarcoding techniques to study cryptic fauna challenge the universality of such gradients. While surveying lesser-known areas of the Pacific Ocean in 2023 and 2024, the National Geographic Society's Pristine Seas program collected ~570 environmental DNA samples from tropical coral reefs across six regions spanning 70° of longitude. We amplified and sequenced these samples using a 12S fish primer set as well as a novel 28S primer set to assess invertebrate and algal biodiversity. Dissimilarity analyses of fish, invertebrate, and algal communities largely reflected geography with sites near the center of the Coral Triangle separating from those farther away. Richness per sample was higher inside the Coral Triangle for certain fish families, but higher outside for others, with no clear gradient overall. Some invertebrate and algal groups showed declining richness moving eastward, but this pattern was not observed for a few key groups. These findings highlight the biodiversity of minimally impacted Pacific coral reefs and demonstrate the potential of environmental DNA to uncover large-scale biogeographic patterns across the tree of life.

【P-41】

海藻養殖による炭素固定能の評価に向けた環境中のオキナワモズク由来DNAの種特異的な検出・定量系の開発

Developing a method to detect and quantify *Cladosiphon okamuranus* DNA from the environment to assess the carbon fixation potential of seaweed farms

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近年、海藻養殖は光合成による二酸化炭素除去効果が期待され、養殖現場における二酸化炭素固定能の正確な評価が求められている。この評価にあたっては、残存有機炭素が養殖海藻由来であることが重要となり、養殖海藻由来DNAはその指標として有用であると考えられる。そこで本研究では、食用もずくの主要養殖種であるオキナワモズク（*Cladosiphon okamuranus*）のDNAを環境試料から検出・定量する系の確立を試みた。核ゲノム中およびミトコンドリアゲノム中の配列を標的としたプライマーセットを複数設計し、オキナワモズクおよびその近縁種の藻体DNAを用いて種特異的増幅の可能なセットを選抜した。さらに標的配列の正確な定量のために加水分解プローブを設計し、デジタルPCRによる定量精度と検出限界を検討した。発表では、これらの検出系を用いた実際の野外サンプルでの定量結果についても報告する。

Seaweed aquaculture is expected to contribute to carbon dioxide removal through photosynthesis, necessitating accurate assessment of its carbon dioxide fixation capacity in aquaculture sites. Determining the amount of residual organic carbon derived from farmed seaweed in marine areas is crucial, and DNA from farmed seaweed is considered a useful indicator. We developed a system to detect and quantify DNA from okinawa-mozuku (*Cladosiphon okamuranus*), a major cultivated species, in environmental samples. Multiple primer sets targeting sequences within the nuclear and mitochondrial genome were designed. The primer sets were screened for their ability to specifically amplify the *C. okamuranus* DNA in PCR using genomic DNA samples extracted from *C. okamuranus* and its related species. For accurate quantification of the target sequence copy numbers, we established a method using digital PCR with hydrolysis probes and evaluated the detection limit and quantification accuracy. We will present the current results on environmental samples using our method.

【P-42】
環境 DNA を用いた河川における自然再生事業の評価

Impact assessment of nature restoration projects in rivers using environmental DNA

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人間活動による生物多様性の損失や生態系サービスの劣化が懸念されている。その回復のためには自然環境の保全・再生のための施策とともに、これらの施策が生態系に与えた影響を適切に評価することが求められる。特に生物相への影響の把握は生態系の状態評価において中心的な役割を担い、そこで環境 DNA 技術が重要な役割を果たすことが期待されている。本研究では、神奈川県内を流れる 2 河川（相模川・酒匂川）において、当該河川で実施された自然再生事業が生態系に与えた影響を環境 DNA の多地点調査をもとに評価した。具体的には、水生昆虫・両生類・魚類を対象にした環境 DNA メタバーコーディングの結果を自然再生事業実施地点と非実施地点の間で比較することで、自然再生事業が生物多様性に及ぼす効果を定量的に評価した。特に、環境データから予測される生物多様性指標と実測値を比較することにより、自然再生事業の効果を多角的に評価した。

Biodiversity loss and the degradation of ecosystem services caused by human activities have become a major concern. To address these issues, not only are measures for the conservation and restoration of natural environments required, but it is also essential to properly evaluate their impacts on ecosystems. In particular, assessing ecosystem responses plays a vital role in evaluating ecological status, and environmental DNA (eDNA) technology is expected to serve as a valuable tool in this context. In this study, we evaluated the impacts of nature restoration projects on ecosystems in two rivers in Kanagawa Prefecture, Japan (the Sagamigawa and Sakawagawa Rivers), based on a multi-site eDNA survey. Specifically, we compared the results of eDNA metabarcoding targeting aquatic insects, amphibians, and fish between restored and control sites, thereby quantitatively assessing the impacts of restoration projects on biodiversity. In addition, by comparing biodiversity indices predicted from environmental data to observed values, we conducted a multifaceted assessment of the ecological outcomes of the restoration projects.

【P-43】
都内淡水域における水生生物を対象とした環境 DNA 調査

Environmental DNA-based survey of aquatic organisms in freshwater bodies in Tokyo

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都内淡水域において環境 DNA・採集調査を実施し，保護上重要な種の生息・生育状況，生息・生育環境の変化等を把握した。魚類では，環境 DNA 調査で検出された種類数が採集された種類数を包括していたことから，環境 DNA 調査は生息魚種の把握に有効であると考えられた。一方，水生植物と底生動物では採集調査で確認された種類の中には環境 DNA 調査で検出されない種類が存在したことから，多系統を含むこれらの分類群では網羅的な検出は現時点では難しいと判断された。魚類のうち，東京都レッドリスト掲載種であるスナゴカマツカの環境 DNA が検出された地点はごくわずかであった。ドジョウ在来系統の環境 DNA が検出された地点では，ドジョウ外来系統の環境 DNA と同所的に確認されることがほとんどであり，また，外来系統のみの確認地点も多かった。国内外来魚であるカワムツ，タカハヤ，カワヨシノボリの環境 DNA も広範囲において確認された。

Environmental DNA (eDNA) sequencing and organism collection surveys were conducted in freshwater bodies in Tokyo to understand the distribution and habitat preferences of species of conservational interest and identify changes in their habitats. The eDNA survey identified all fish species captured in the collection survey, suggesting that eDNA analysis can effectively assess fish species diversity. However, some species of aquatic plants and benthic invertebrates confirmed to be present via the collection survey were not detected via the eDNA survey, indicating that for these taxonomic groups, which include multiple lineages, comprehensive detection is not currently achieved using eDNA.eDNA from the Tokyo Red List species *Pseudogobio polystictus* (Sunago-kamatsuka) was detected at only a few sites. At locations where eDNA from native lineages of *Misgurnus anguillicaudatus* (Dojo loach) was detected, the eDNA of non-native lineage was also commonly found. At many other sites, only eDNA from non-native lineage was observed. In Tokyo, eDNA from domestical non-native fish species including *Nipponocypris temminckii* (Kawamutsu), *Rhynchocypris oxycephalus* (Takahaya), and *Rhinogobius flumineus* (Kawayoshinobori) were widely detected.

【P-44】

宮崎県清武川水系における環境 DNA 移流モデルと流量データを用いた河川生物量推定手法の検討

Development of a riverine biomass estimation method using environmental DNA transport model and discharge data in the Kiyotake River

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河川における eDNA を用いた生物調査は，流量の違いにより eDNA が濃縮・希釈され，正確な存在量推定が困難である．本研究では，カマツカ（ *Pseudogobio esocinus* ）を対象に，宮崎県清武川流域において，eDNA 濃度・河川流量・eDNA 放出率を組み合わせて，生物量を推定する手法を提案する．特に，非閉鎖性水域である河川において流下の影響を受けやすい eDNA の空間的変化を把握するために，移流モデルを用いて eDNA の流下・減衰プロセスも評価に取り入れた．eDNA に基づく生物量推定の有用性評価のために，たも網を用いた採捕調査結果との比較を行った．結果として，eDNA 濃度は採捕調査で多くの個体数を捕獲した地点で移流を考慮しても高い値を示し，正の相関関係が確認された．したがって，eDNA を用いた推定バイオマスはカマツカの存在量を反映しており，その有効性が示された．

Environmental DNA (eDNA) surveys in rivers are influenced by flow variations, which can dilute or concentrate eDNA and make abundance estimation difficult. This study proposes a method for estimating the biomass of the *Pseudogobio esocinus* in the Kiyotake River basin, Miyazaki, Japan, by combining eDNA concentration, river discharge, and species-specific eDNA shedding rates. Because rivers are open systems, downstream transport strongly affects eDNA, making precise quantification challenging. To capture spatial changes, we incorporated a transport model to evaluate downstream transport and degradation processes of eDNA. To assess the effectiveness of eDNA-based biomass estimation, we compared results with capture surveys using hand nets. The results showed that eDNA concentrations were consistently high at sites where many individuals were captured, even after accounting for transport, and a positive correlation with capture data was confirmed. These findings indicate that biomass estimated from eDNA reflects the abundance of *P. esocinus* and demonstrates the usefulness of eDNA for biomass estimation in riverine environments.

【P-45】

環境 DNA 分析による別府湾堆積物を用いた 1900 年前までわたる生物群集の長期復元

Long-term reconstruction of biological communities over the past 1900 years from Beppu Bay sediments using environmental DNA analysis

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本研究では、別府湾の海底堆積物コアを試料とし、魚類、哺乳類、真核生物に対応したユニバーサルプライマーを用いて環境 DNA メタバーコーディング分析を実施した。その結果、堆積物から約 1,900 年前までに遡り DNA 断片が検出され、環境 DNA が堆積物中で長期保存されており、過去の生物相を調査する上で有用であることが示唆された。魚類ではコイ科やサケ科が近代で多く検出され、近隣の養殖の影響と考察した。哺乳類ではカワネズミが西暦 200 年頃から近代に至る層まで継続的に検出され、河川由来の DNA が海底に堆積していたと考えられた。さらに、海藻、プランクトン、ホヤ類など多様な海生生物の分類群も同定され、堆積物が陸域と海域双方の生物情報を反映していることが確認された。別府湾の海底堆積物解析により、生物多様性の長期的変動や人為的影響を明らかにする有効な手段であることが示唆された。

In this study, sediment cores from Beppu Bay were used as samples, and environmental DNA (eDNA) metabarcoding analyses were conducted with universal primers targeting fishes, mammals, and eukaryotes. As a result, DNA fragments were detected dating back approximately 1,900 years, suggesting that eDNA can be preserved in sediments over long timescales and is useful for reconstructing past biotic communities. For fishes, members of the families Cyprinidae and Salmonidae were frequently detected in the modern layers, which was interpreted as being influenced by nearby aquaculture activities. For mammals, the water shrew was consistently detected from around 200 CE to recent layers, implying that river-derived DNA had accumulated in the marine sediments. Furthermore, a wide range of marine taxa such as seaweeds, plankton, and ascidians were identified, confirming that the sediments reflect biological information from both terrestrial and marine environments. These results indicate that the analysis of Beppu Bay sediments provides an effective means to reveal long-term biodiversity dynamics and anthropogenic impacts.

【P-46】
環境 RNA による沿岸生態調査における飼料由来偽陽性の低減

Environmental RNA reduces feed-derived false positives in coastal field surveys

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沿岸域の環境 DNA メタバーコーディングは生物多様性評価に有用だが、養殖飼料など外来核酸により偽陽性が生じる課題がある。本研究では、養殖飼料と湾内水を並列で解析し、環境 DNA と環境 RNA の検出傾向と空間分布を比較した。飼料由来の核酸は水中でも検出され得るが、環境 RNA は環境 DNA に比べ検出数が少なく、より局所的な検出にとどまる傾向が示された。これは環境中での RNA の分解が速いことと一致し、環境 RNA 導入により飼料由来の偽陽性を低減できる可能性がある。ただし完全な排除は困難であり、qRT–PCR などの定量手法や流動・分散モデルとの併用により、真の生息情報と核酸流入をより正確に識別できると考えられる。

Environmental DNA (eDNA) metabarcoding is widely used for aquatic biodiversity monitoring, but interpretation can be confounded by extraneous nucleic acids introduced by human activities such as aquaculture. We examined whether environmental RNA (eRNA) metabarcoding can mitigate feed-derived false positives in coastal surveys. Commercial aquaculture feeds and water samples were collected at multiple locations around a cultured bay, including sites at and away from the aquaculture facilities, and analyzed in parallel using standard DNA- and RNA-based metabarcoding followed by high-throughput sequencing and taxonomic assignment to reference databases. Focusing on overall detection patterns rather than detailed species lists, we compared DNA- and RNA-derived detections and their spatial distributions relative to the farming sites. Both DNA and RNA originating from feeds were detectable in nearby water, but RNA-based detections in environmental samples tended to be fewer and more spatially constrained than DNA-based detections. These qualitative differences are consistent with more rapid degradation and lower environmental persistence of RNA, indicating that eRNA metabarcoding can reduce the likelihood of feed-derived false positives in coastal biodiversity assessments. While eRNA improves discrimination between true organismal presence and nucleic-acid influx, it may not fully eliminate contamination-driven detections. We therefore suggest using eRNA alongside eDNA surveys and, where appropriate, complementing them with quantitative molecular assays and environmental transport or degradation analyses to increase confidence in occurrence inferences in areas influenced by aquaculture.

【P-47】
深海での展開が可能な大流量 eDNA サンプラーの開発と評価

Development and evaluation of a high-throughput, deep-sea deployable eDNA Sampler

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深海域における環境 DNA（eDNA）の採取は、技術的な制約によりこれまでの取り組みは限定的である。本研究では、6,000m までの深海環境で使用可能な eDNA サンプラーを、3D プリント技術を用いて製作した。本装置は 300 ～ 500 ml/min 程度の流量で海水をろ過し、ステリベクスフィルタ等に eDNA を濃縮・採取できる。ろ過後にはフィルタに保存試薬が自動導入される。2025 年 5 月には深海用フリーフォール型無人探査機「江戸っ子 1 号」に搭載して水深約 1500m の深海環境に 2 台のサンプラーを投入し、海底近傍の eDNA 採取を実施した。海水の濾過量が MiFish 分析結果に及ぼす影響を見積もるため、サンプルの採取量は約 5L と 20L となるよう設定した。ここでは、主に深海用大流量 eDNA サンプラーの概要と、深海環境から得られたサンプルの比較分析結果について報告する。

Sampling of environmental DNA (eDNA) in the deep sea has been limited due to technical constraints. In this study, we developed a novel eDNA sampler, designed for deployment at depths of up to 6,000 m, using 3D printing technology. The device filters seawater at a flow rate of approximately 300 ～ 500 ml/min and concentrates eDNA onto Sterivex filters or equivalent cartridges. After filtration, preservation reagents are automatically introduced into the filter unit. In May 2025, two samplers mounted on the free-fall type deep-sea lander “Edokko No.1” were deployed at a depth of approximately 1,500 m to collect eDNA samples near the seafloor. To evaluate the effect of filtration volume on downstream MiFish analysis, the total filtered seawater volume was set to approximately 5 L and 20 L, and the resulting samples were compared. In this presentation, we report an overview of the newly developed deep-sea eDNA sampler and comparative analyses of the samples obtained from deep-sea environments.

【P-48】

Thermal traps constrain community reorganization under global warming

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Ecosystem persistence under climate warming is associated with how communities reorganize. While faster warming rates are often attributed to accelerated reorganization, asymmetric warming in seasonal extremes may instead constrain reorganization, yet these mechanisms remain poorly understood. Using a five-year eDNA time series from Japanese coastal waters, we demonstrate that asymmetric warming of seasonal water temperature extremes creates "thermal traps" that confine the tropicalization of cold-water communities. This constraint arises because cold-water communities, already confined by narrow Thermal Safety Margins (TSMs) near their lower limits, face intensified stress: winter minimum temperature decline poleward more steeply than summer maximum temperature and rise more slowly under ongoing warming. These communities appear seasonally stable, but can face abrupt biodiversity loss once warm TSMs are exhausted. In contrast, warm-water Kuroshio communities close to their upper limits temporarily buffer warming stress through rapid tropicalization and continual turnover. Together, these divergent dynamics reveal that apparent stability can signal entrapment in thermal traps, underscoring the need to recognize such traps when assessing vulnerability and safeguarding marine biodiversity in a rapidly warming world.

【P-49】
イシガイ類メタバーコーディングの改良

Improvements to Metabarcoding for Unionidae

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イシガイ類は淡水生態系において重要な役割を果たすが、その多様性の把握や生息状況の評価には効率的かつ網羅的な手法が求められる。我々は昨年度、環境 DNA を用いた定量メタバーコーディング法を構築し、その成果を報告した。しかし、ヌマガイにおいては定量 PCR との比較でコピー数が過小評価される傾向がみられ、さらに、経験的にマツカサガイの検出率が低いことが課題であった。本研究では、九州北部産イシガイ類の 16S 領域塩基配列を精査し、プライマー配列との間に存在する 1 ～ 2 塩基のミスマッチが影響している可能性に着目した。そこで、これらのミスマッチをカバーするプライマーカクテルを設計し、従来プライマーと比較解析した。その結果、マツカサガイの検出感度が顕著に改善され、定量性についても向上が確認された。本改良法は、イシガイ類の環境 DNA 解析の精度向上に寄与し、淡水生態系モニタリングの高度化に資する可能性が高い。

Unionidae freshwater mussels play a vital role in freshwater ecosystems as bioindicators and key components of biodiversity. Accurate and comprehensive methods are required to assess their diversity and population status, yet methodological challenges remain. We previously developed a quantitative metabarcoding approach for Unionidae using environmental DNA (eDNA) and reported its utility. However, two issues became apparent: first, for *Sinanodonta lauta*, copy numbers were consistently underestimated compared with quantitative PCR results; second, when using U16S primers, the detection rate of *Pronodularia japanensis* was empirically low. To address these limitations, we examined the nucleotide sequences of the 16S region of Unionidae species inhabiting northern Kyushu and assessed potential mismatches between these sequences and the U16S primer set. Our analysis indicated that 1–2 base mismatches may have reduced amplification efficiency. Therefore, we designed a primer cocktail specifically covering these mismatches and conducted comparative analyses using identical samples with both the conventional primer set and the newly designed cocktail. The new cocktail significantly improved the detection sensitivity of *P. japanensis* and showed enhanced quantitative performance for *S. lauta*. These results demonstrate that even slight mismatches can critically influence the accuracy of metabarcoding assays, and that tailored primer design can substantially improve both sensitivity and quantitativeness. This methodological improvement will contribute to more precise eDNA-based monitoring of Unionidae and is expected to advance the accuracy and sophistication of freshwater ecosystem assessment and conservation practices.

【P-50】
マンソン住血吸虫の環境 DNA の検出感度向上に向けた方法論的進展

Methodological advances for sensitive detection of *Schistosoma mansoni* eDNA

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Schistosoma mansoni, the etiological agent of intestinal schistosomiasis, infects humans through exposure to freshwater containing cercariae released by infected intermediate host snails. Identifying contaminated water bodies is critical for targeted disease control. However, traditional snail surveillance is costly, labor-intensive, and impractical for large-scale or real-time monitoring. Rapid shifts in snail populations due to rainfall or land-use changes further complicate timely identification of transmission sites. Environmental DNA (eDNA) analysis has already been established as a promising tool for detecting *S. mansoni* in aquatic environments, but its broader application is limited by issues such as low detection sensitivity, filter clogging in turbid water, and challenges in securing stable electricity and laboratory facilities in endemic regions. To address these challenges, this study conducted distribution surveys in a schistosomiasis-endemic region along the shores of Lake Victoria, Kenya, and compared different methodologies for eDNA analysis.As a key achievement of this study, we demonstrated that targeting a retrotransposon region in real-time (qPCR) analysis of *S. mansoni* eDNA increased detection sensitivity by approximately 100-fold compared to the commonly used qPCR assay targeting the mitochondrial COI region. This highly sensitive assay enabled quantitative analysis of *S. mansoni* eDNA, enhancing its potential for rapid, large-scale surveillance. Furthermore, to overcome field-related operational constraints, we evaluated two electricity-free filtration techniques, gravity filtration and the QuickConc methods. Water samples were collected and processed on-site using both methods, followed by qPCR detection. The QuickConc method exhibited superior filtration capacity and higher detection rates compared to gravity filtration, demonstrating its suitability for robust eDNA-based surveillance in resource-limited tropical settings.

【P-51】

環境 DNA 分析による農作物害虫の発生量把握の検討

Evaluation of environmental DNA analysis for assessing crop pest abundance

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農作物の質と収量の維持には農作物害虫の早期発見と対策が不可欠である。しかし農業従事者の高齢化等で従来の目視による農作物害虫の早期発見、同定は困難になりつつある。我々はこれまでに農作物の葉や果実の表面から環境 DNA を回収して PCR することで農作物害虫の DNA を検出できることを確認した。環境 DNA の中に含まれる農作物害虫の DNA を定量しその発生個体数も推測できれば病害虫の対策を行う上で重要な情報になる。そこで本研究では高知大学内にある施設園芸ハウス内のタバココナジラミ *(Bemisia tabaci)* の個体数を目視で観察して、同時に葉に残された DNA 濃度の関係を調査した。具体的にはタバココナジラミの個体数と、作物の葉から回収されたタバココナジラミの DNA 濃度を定量 PCR (qPCR) で定量して比較した。

Early detection and control of crop pests are essential for maintaining crop yield and quality. However, visual inspection is becoming increasingly difficult due to the aging agricultural workforce. Molecular approaches using environmental DNA (eDNA) provide promising alternatives. We have previously confirmed that pest DNA could be collected from leaf and fruit surfaces and detected by PCR. Quantifying pest DNA from eDNA may also allow indirect estimation of pest abundance, offering valuable information for timely control. Here, we examined the relationship between whitefly (*Bemisia tabaci*) abundance and its DNA residues on crop leaves in a greenhouse at Kochi University. Whitefly individuals were counted visually, and DNA left on leaves was collected, extracted, and quantified by quantitative PCR (qPCR). The correlation between observed whitefly numbers and recovered DNA concentration was then evaluated.

【P-52】
樹幹流中に含まれる真菌類の DNA メタバーコーディング解析

Fungal diversity in tree stemflow revealed by DNA metabarcoding

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樹上には多様な植物や菌類が生育し複雑な生態系が形成されるが、調査の困難さからその種多様性の理解は依然として乏しい。演者らは環境 DNA メタバーコーディングを、樹皮を流下する雨水（樹幹流）に適用し、樹上生物の多様性を調査する手法の開発を進めてきた。本研究では、これまで地衣類を対象としてきた本手法を真菌群集へと拡張し、その有効性を検討した。屋久島の温帯林において 5 樹木から 3 日間にわたり計 15 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、菌類ユニバーサルプライマー（ITS86F/ITS4）を用いて解析した。その結果、樹幹流中には子嚢菌門を中心とした真菌群集が検出され、多くの未知系統が含まれることが示唆された。また樹木や採水日ごとに異なる群集が検出され、樹幹流により樹上真菌群集の時空間的変動を把握できる可能性が示された。

Forest canopies host diverse plants and fungi that form complex ecosystems. However, their biodiversity remains poorly understood due to difficulties in direct sampling, highlighting the need for rapid and non-invasive survey methods. We have been developing a monitoring approach that applies environmental DNA (eDNA) metabarcoding to stemflow, the rainwater that drains along tree bark, as a proxy for canopy-associated organisms. In this study, we extended our previous application of this method from lichens to fungal communities and evaluated its potential for biodiversity surveys. Stemflow samples were collected from five trees over three days in a temperate forest of Yakushima Island, Japan, resulting in a total of 15 samples. DNA was extracted from filters after gravity filtration, and fungal diversity was analyzed using the fungal universal primer pair (ITS86F/ITS4). The results revealed that stemflow contained fungal communities dominated by Ascomycota, while also including a substantial proportion of unclassified taxa. Furthermore, distinct fungal assemblages were detected among tree individuals and across sampling dates. These findings suggest that stemflow can capture both spatial and temporal variation in canopy-associated fungal communities. Although further replication and validation are needed, our study demonstrates the potential of stemflow eDNA metabarcoding as a complementary tool for assessing fungal diversity in forest canopies.

【P-53】
eDNA によるシイタケ菌床の害虫検出手法の開発

Developing an eDNA-based method for detecting pests on Shiitake mushroom substrates

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本研究は、eDNA を利用し、簡便かつ迅速に、シイタケ菌床栽培施設内で発生している害虫ナガマドキノコバエ類（ナガマド類）を検出する手法開発を目的とした。現在日本で流通する国産生しいたけの 9 割以上は、おがくずに米ぬか等を加えた培地で栽培する菌床栽培施設で生産されている。ナガマド類は、周囲環境から栽培施設に入り込み、収量減少や幼虫による異物混入トラブルの原因となる。害虫ナガマド類には、形態が酷似した種が複数あり、適切な防除のため検出・同定の迅速さ、精確さが求められる。本研究では、栽培施設設置後 2 カ月以上の菌床 3 個を対象に、菌床に水をかける、又は浸す処理により、表面に残存した害虫類の eDNA 回収を試みた。採取水のろ過処理、沈殿処理、上澄み液のエタノール沈降の 3 処理において、アンプリコンシーケンス解析により害虫類の検出効率を比較した結果、沈殿処理により得られた沈殿汚泥がもっとも検出効率が高かった。

We here aimed at developing a simple, rapid method for detecting several species of pest fungus gnats (*Neoempheria*, Mycetophilidae) in shiitake mushroom cultivation facilities using eDNA. Currently, over 90% of the fresh shiitake mushrooms being produced and distributed in Japan are grown in facilities using sawdust-based substrates mixed with rice bran and other materials. Adults of *Neoempheria* species are thought to invade the facilities from outdoor environments and the larvae inhabit on the substrate and fruiting bodies, causing yield reductions and contamination issues. Because *Neoempheria* pests include morphologically similar multiple species, rapid and accurate detection is required to control them. We attempted to recover eDNA from pest insects remaining on the surface of substrate blocks by washing or immersing them in water at more than 2 months after installation to the cultivation facilities. Amplicon sequencing analysis suggested that the detection of *Neoempheria* species would be higher for the extracts from sediment obtained through sedimentation of washing water compared to those from filtering or ethanol precipitation of the supernatant.

【P-54】

マルチプレックス PCR を用いた環境 DNA メタバーコーディングによる多分類群同時検出とその現場適用性

Simultaneous multi-taxa detection by multiplex PCR-based environmental DNA metabarcoding and its field applicability

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従来の生物調査（捕獲・観察）は、分類群ごとに調査手法や同定基準が異なり、多大な労力と専門的スキルを要する。一方、環境 DNA メタバーコーディングは共通の調査手順で複数分類群を網羅的に検出できるが、それらの分析プロセスは分類群ごとに個別の処理が必要であり、コスト効率の低さが実用化の制約となっている。

本研究では、この課題を克服するため、マルチプレックス PCR を環境 DNA メタバーコーディングに導入し、複数分類群を同時に検出する新しい手法を開発した。さらに、国土交通省の「河川水辺の国勢調査」で対象とされる 11 分類群を対象に試行調査を行い、従来型調査との比較によって現場適用性を検証した。本成果は、環境 DNA 分析のコスト効率と実用性を飛躍的に高め、生物多様性モニタリングの高度化に資するものである。

Conventional biological surveys based on capture and observation require different methodologies and identification criteria for each taxonomic group, demanding substantial effort and professional skills. In contrast, environmental DNA (eDNA) metabarcoding enables comprehensive detection of multiple taxa using a common workflow. However, the analytical process still requires separate processing for each group, and the low cost-efficiency has posed a major limitation to its practical application.

To address this issue, we introduced multiplex PCR into eDNA metabarcoding and developed a novel approach for the simultaneous detection of multiple taxa. We further conducted a pilot survey targeting 11 taxonomic groups included in the “National Census on River and Dam Environments” and evaluated the field applicability of the method by comparing the results with those of conventional surveys. Our findings demonstrate that this approach can markedly improve the cost-efficiency and practicality of eDNA analyses, thereby advancing biodiversity monitoring.

【P-55】
陸上の環境 DNA を効率的に捕集する Koro-rin 法の開発

Development of the Koro-rin method for efficient collection of terrestrial environmental DNA

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環境 DNA（eDNA）のサンプリング手法は水域で多く研究されている一方、陸上環境での応用は限られ、標準化は十分に進んでいない。そこで、回転体と不織布を組み合わせたサンプラーを地表で転がして eDNA を回収する「Koro-rin 法」を開発した。野生動物が生息する里山環境で Koro-rin 法による地表サンプリングを実施し、MiBird/MiMammal プライマーを用いたメタバーコーディングによって周年解析した結果、鳥類・哺乳類を計 53 種検出し、カメラで記録された種の 92%と一致した。また、季節性の鳥類では DNA 検出のタイミングが実際の飛来時期と一致し、時系列モニタリングにも有用であることが示唆された。Koro-rin 法は水のない場所を含む多様な地表に適用可能であり、水域での手法と組み合わせることで生態系全体の包括的解析が期待される。

Environmental DNA (eDNA) sampling has been well establisedin aquatic environments, but applications in terrestrial environments remain limited and lack standardization. We developed the “Koro-rin method,” a sampler combines a rotating body withnon-woven fabric to collect eDNA by rolling on the ground surface. Using this method, we conducted year-around sampling in a satoyama landscape and anlyzed the samples with DNA metabarcoding with MiBird/MiMammal primers. In total, 53 bird and mammal species were detected, and 92% of the species recorded by cameras were also detected by the eDNA survey. For seasonal birds, detection timing of eDNA detection corresponded with actual arrival periods, demonstrating potential for time-series monitoring. The Koro-rin method can beapplied to diverseground surfaces, including waterless areas. Combined with methods for aquatic environments, it offers poweful tool for enhancing our understanding of the whole ecosystem.

【P-56】

野外環境水からの細胞核回収法の確立および分析手法の最適化：交雑種検出への応用

Establishment of nuclear recovery methods from field environmental water and optimization of analytical techniques: Application to hybrid detection

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従来の環境 DNA 分析では主に断片化した DNA が対象であり、取得できる生物情報は限られる。特に、交雑種の検出には２種に由来するアレルを DNA 断片として検出した場合、２種の同所的生息と区別ができない問題が生じる。むしろ DNA 抽出を介さず細胞内（核内）DNA をデジタル PCR することで、両親由来の対立遺伝子の同時検出が可能となり、同所的分布と判別が可能であると考えられる。そこで本研究では、環境水中から核構造を保持したまま回収する方法を検討した。核内 DNA を効率的に回収できるろ過孔径の検討では、ナイロンメンブレンフィルターでろ過、デジタル PCR を実施し、孔径 30.0㎛の回収効率が高いことが確認された。さらに、濾過法と遠心法を組み合わせることでより多くの核の回収効率向上が期待できる。本手法確立により、野外での交雑による遺伝的浸透の非侵襲的検出が可能となり、保全管理に向けた基盤技術となると期待される。

Conventional environmental DNA (eDNA) analysis primarily targets fragmented DNA, limiting the biological information obtainable. Specifically, detecting hybrid species poses a challenge: when alleles from two species are detected as DNA fragments, they cannot be distinguished from sympatric occurrence of the two species. Rather than extracting DNA, conducting digital PCR on intracellular (intranuclear) DNA enables simultaneous detection of alleles from both parents, allowing differentiation from sympatric distribution. Therefore, this study investigated methods for recovering nuclei from environmental water while maintaining nuclear structure. To determine optimal filtration pore sizes for efficient nuclear DNA recovery, samples were filtered through nylon membrane filters and analyzed by digital PCR. Results confirmed that a pore size of 30.0 ㎛ achieved high recovery efficiency. Furthermore, combining filtration and centrifugation methods is expected to enhance nuclear recovery efficiency. The establishment of this methodology will enable non-invasive detection of genetic introgression through hybridization in field environments, serving as foundational technology for conservation management.

【P-57】

野外における生態－進化フィードバック解明に向けた樹上環境 DNA アプローチの活用

Utilization of an arboreal eDNA approach for elucidating the eco-evolutionary feedback in natural conditions

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生態的特性（群集構造や個体群動態）と進化（遺伝子頻度の変化）の間の循環的相互作用は生態－進化フィードバック（EEF）と定義されている。EEF は種・遺伝的多様性の維持メカニズムの解明に繋がる重要な概念とされ、様々なモデル系で研究されてきたが、野外実態の理解は十分でない。その理由として、EEF の観測に必要な異なる階層（遺伝子～生態）の経時的データの取得が従来の調査手法では非常に困難なことが挙げられる。

本研究では、ヤナギ樹上の節足動物群集とヤナギルリハムシ（P. versicolora）をモデル系として、環境 DNA アプローチによる群集構造とハムシの進化の観測を二年間毎週行った。本モデルは、節足動物群集とハムシの餌嗜好性の進化が相互に作用することが分かっている。両時系列データを用いた因果推定の結果、異なる時間差で双方向の相互作用が推定された。また、それらの時間差は生態学的変化と一致していた。

Eco-evolutionary feedback (EEF) is defined as the cyclical interactions between ecology (e.g., community structure and population dynamics) and evolution (the change in allele frequencies). EEF is one of key concepts for the mechanisms that shape species and genetic diversity in ecological communities. However, empirical evidence and consequences of EEF under natural conditions are poorly understood. This is because it is difficult to obtain time-series data sets across multiple levels of biological organization (i.e., from molecule to ecosystem) using the conventional method. Here, we focus on an eDNA approach as a possible way to track ecological and evolutionary dynamics simultaneously.

In our study, we utilized the arthropod community on the willow trees and the willow leaf beetles (*Plagiodera versicolora*) as a field model system. Previous studies have shown that arthropod community structure and evolution of feeding behavior (i.e., specialization/ generalization) of the leaf beetle influence one another reciprocally. The underlying SNP maker for the beetle's feeding trait was also developed. We collected eDNA samples once a week for two years and performed multiplexing NGS analysis using metabarcoding primers and trait-associated SNP primers. Then, a causality test, using two years of weekly community and evolutionary time-series data, was performed. We found that bidirectional causal relationships between community dynamics and evolutionary dynamics in different time lags. These time lags made sense in term of ecological changes in the communities and natural history of the leaf beetle. In this presentation, we will discuss how eDNA approach is promising toward an understanding EEF in nature.

【P-58】

森林林冠層の生物多様性把握に向けた樹幹流を利用した環境 DNA 解析の適用可能性検討

Stemflow eDNA as a window into forest canopy biodiversity: A case study on lichens

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森林の林冠層には植物や菌類を中心とした極めて高い生物多様性が存在しており、その網羅的把握には広域かつ継続的なモニタリングが不可欠である。しかし、高所に位置する林冠層はアクセスが困難で、従来の目視や直接採取に依存した調査法には限界がある。そこで我々は、地上から非侵襲的かつ低コスト・低労力で林冠層の生物多様性を把握する新規モニタリング法として、樹幹流を利用した環境 DNA 解析の適用可能性を検討した。千葉県鴨川市にある東大演習林のアカガシ優占林で高木 4 本から計 8 回の樹幹流を回収し、重力濾過後に DNA を抽出して地衣類の環境 DNA メタバーコーディング解析を行った。その結果、8 試料中 4 試料から地衣類 DNA を検出した。調査木では高所にのみ地衣類の生育が確認されていることから、本手法により林冠層の生物多様性を把握できる可能性が示唆された。

Forest canopies harbor exceptionally high biodiversity, particularly of plants and fungi, and their comprehensive assessment requires broad-scale and continuous monitoring. However, canopy access is difficult, and conventional methods relying on visual observation or direct sampling are limited. We therefore examined the applicability of environmental DNA (eDNA) analysis of stemflow as a novel, non-invasive and cost-effective method to monitor canopy biodiversity from the ground. In a Quercus acuta-dominated forest at The University of Tokyo Chiba Forest, we collected a total of eight stemflow samples from four tall trees. After gravity filtration, DNA was extracted and subjected to eDNA metabarcoding analysis of lichens. Lichen DNA was detected in four of the eight samples. Because lichens growth on the sampled trees were confirmed only in the upper part of the trunk, our results suggest that this method can provide valuable information on canopy biodiversity.

【P-59】

森林における環境 DNA 分析を用いた生物相調査ー河川水サンプリングと水かけ法ー

Biota survey using environmental DNA analysis in forests from river water and tree leaves

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生物モニタリング法として利用されている環境 DNA 分析は、水生生物から陸生生物への応用が進んでいる。空気中に浮遊する陸生動物の環境 DNA は、植物の葉や枝に付着すると考えられ、それらを洗い流すことで空気由来の環境 DNA の検出が試みられている。本研究は、森林内河川にて魚類、哺乳類、鳥類の検出を目的に、河川水を採水した。加えて、哺乳類と鳥類を対象に、葉や枝に付着した環境 DNA を捉えるため水で洗い流して回収する水かけ法を実施した。19 地点の河川水と 7 地点の水かけ法でサンプリングを行い、分類群ごとに環境 DNA メタバーコーディング分析を行った。結果、3 科 3 属 30 種が検出され、哺乳類は河川水で 6 分類群、水かけ法で 5 分類群、鳥類はそれぞれ 6 分類群と 14 分類群が検出され、鳥類では水かけ法において多くの分類群が検出された。この結果は、森林内の生物相調査は分類群に応じた適切なサンプリング方法が必要だと示唆される。

The use of environmental DNA (eDNA) analysis as a biological monitoring technique is progressively expanding from aquatic to terrestrial organisms. The eDNA of terrestrial animals suspended in the air is thought to adhere to plant leaves and branches, and attempts to detect airborne eDNA by washing these surfaces have been actively explored. This study collected river water samples from the forest streams to detect fish, mammals, and birds. In addition, for mammals and birds, a water-washing method was applied to recover eDNA attached to leaves and branches. Sampling was conducted at 19 sites for river water and at 7 sites for the water-washing method, and eDNA metabarcoding analyses were conducted separately for each taxonomic group. As a result, a total of 36 taxa were detected, comprising 3 families, 3 genera, and 30 species. Mammals were represented by 6 taxa in river water and 5 taxa in the water-washing method, while birds were represented by 6 and 14 taxa, respectively, with more bird taxa detected using the water-washing method. These findings indicate the importance of taxon-specific sampling methods for effective biodiversity surveys in forest ecosystems.

【P-60】
山地河川水の環境 MIG-seq 分析による集水域内優占樹種の遺伝的多様性評価

Assessment of genetic diversity of dominant trees from river water using MIG-seq analysis of environmental DNA (eMIG-seq)

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森林内を流れる河川水の環境 DNA からは、水生生物の情報だけでなく、集水域内に分布する樹木の遺伝的情報も得られる可能性がある。また従来の種の在不在情報だけでなく、種内の多様性情報も含まれる。そこで本研究では、ゲノムワイド SNP 情報の取得法として MIG-seq 法を用い、河川水の環境 DNA 分析による遺伝的多様性評価法の開発を行った。まず室内実験用に、落葉広葉樹の葉と水を入れた模擬河川水を作製し、その遺伝的多様性の再現性を検証した。次に野外実験用に、冷温帯落葉広葉樹林を流れる河川水から環境 DNA を採集し、主要構成樹種であるブナの遺伝的多様性の把握を試みた。さらに、河川水中の微量な植物 DNA に対しターゲットキャプチャー法を適用し、対象種の DNA を選択的に濃縮し効率的に情報を取得した。この手法は環境 DNA から得られる様々な生物種に適用可能な手法であり、この分野に大きな変革をもたらす可能性がある。

Environmental DNA (eDNA) from river water flowing through forests can reflect information about aquatic organisms and it has a possibility that also reflect genetic information about trees distributed within watersheds. In addition, eDNA includes information not only of presence/absence but also of intraspecific genetic diversity. In this study, we aimed to detect genetic diversity information from eDNA of rivers in forests using a genome-wide SNP analysis, MIG-seq. First, we tested artificial water samples by combining leaves of broadleaf trees and water to examine the reproducibility of genetic diversity. Second, we tested river water samples from a cool-temperate broadleaf forest to detect genetic diversity of a dominant tree species, Fagus crenata (beech). Additionally, a target-capture method was applied to enrich low-concentration plant DNA in river water. This method has the potential to bring about major changes in this field because it can be applied to a variety of species obtained from eDNA.

【P-61】
ヘビのフィールドサインからの環境 DNA 検出についての試行

Attempt to detect eDNA from field signs of snake

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ヘビ類は代謝が低く一般的に環境 DNA での検出が難しい分類群であると考えられる。確実なフィールドサインは抜け殻しかなく、在不在の調査も難易度が高い分類群の一つである。そこで、飼育下のアオダイショウを用いて、這跡のぬぐい取り、糞、抜け殻について、どの程度の精度や量の環境 DNA 検出ができるのかを検討した。いずれのサンプルもミトコンドリア DNA の部位を用いた種検出は可能であったが、雌雄判定などができないサンプルもあった。

Snakes are generally considered a taxon difficult to detect via environmental DNA due to their low metabolic rate. The only reliable field sign is shed skin, making them one of the challenging taxa to survey for presence or absence. Therefore, using captive Japanese rat snakes, we investigated the accuracy and quantity of environmental DNA detection achievable from trail swabs, feces, and shed skin. Species detection using mitochondrial DNA sites was possible for all sample types, though some samples did not allow sex determination.

【P-62】

陸域サンプルを用いた環境 DNA 分析による哺乳類相把握の試み ～現地調査結果との比較～

An attempt to assess mammalian fauna through environmental DNA analysis using terrestrial samples: A comparison with conventional field survey results

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環境 DNA による生物相の把握は、現在は主に河川等の水を採取し、その中に含まれる生物由来の DNA を検出する事例が多く、魚類を中心に知見が蓄積されてきた。一方、哺乳類をはじめとした陸棲動物については、環境 DNA の分析対象とするサンプルの取得方法を含めて、事例が少なく知見が乏しい。以上を踏まえて、本件では、陸域で採取したサンプルを基に哺乳類を対象とした環境 DNA 分析を行い、従来の現地調査結果と比較した。

ペイントローラーを用いて陸域でサンプルを採取し、メタバーコーディング法による分析を行った。分析結果について、サンプル採取と同時期に実施した従来手法による調査結果と比較検証を行った結果、今回のサンプリング試料から、哺乳類の DNA が検出され、従来手法による現地調査で確認された種を複数確認することができた。

The use of environmental DNA (eDNA) to understand faunal composition has, to date, primarily focused on collecting water samples from rivers and other aquatic environments, where DNA fragments derived from organisms are detected. This approach has led to a substantial accumulation of knowledge, particularly regarding fish species.On the other hand, in the case of terrestrial animals including mammals, there are few case studies, and knowledge remains limited, including with regard to methods for collecting samples for environmental DNA analysis.

Based on the above, this study conducted eDNA analysis targeting mammals using samples collected from terrestrial environments, and compared the results with those obtained through conventional field surveys.

Samples were collected from land using paint rollers, and analyzed using the metabarcoding method. The results of the analysis were compared and verified against the findings of conventional survey methods conducted during the same period. Mammalian DNA was successfully detected from the collected samples, and multiple species confirmed through conventional field surveys were also identified.

【P-63】

LAMP 法による特定外来生物キョンの検知法の開発

Development of a detection technique for specified invasive species Reeves' muntjac using the LAMP method

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特定外来生物のキョン *Muntiacus reevesi* は本州では千葉県に定着しているが、近年、茨城県等への分布拡大の懸念されている。農作物の食害等による経済被害、キョンの体表に付着したダニが媒介する感染症のリスク等を抑えるためには、早期に侵入を検知して迅速な対策を行うことが非常に重要であるが、環境 DNA 分析による種判別は有力なツールとなる。そこで、迅速かつ安価にオンサイトでも分析が行える LAMP 増幅法を用いたキョンの検知法の確立を目指した。キョンの種特異プライマーを設計し、近縁種との種特異性を確かめた。また、飼育および野生個体の糞からのキョンの判別に成功した。環境水等の野外サンプルを用いた実用性の検証も進めている。

In Honshu Island of Japan, the invasive species, Reeves' muntjac *Muntiacus reevesi*, has been established in Chiba Prefecture. Recently there have been concerns about its distribution spreading to Ibaraki and other prefectures. Early detection of invasions and prompt countermeasures are extremely important to reduce economic damage caused by crop damage and the risk of infectious diseases transmitted by mites attached to the body surface of the mite. In this point, species identification by environmental DNA analysis is a powerful tool. Therefore, we aimed to establish a method for detecting muntjac using the Loop mediated isothermal amplification (LAMP), which is a rapid and inexpensive method that can be performed on-site. We designed a species-specific primer for the muntjac and confirmed its species-specificity with related species. We also succeeded in species identification of muntjac from the feces of captive and wild individuals. Practicality verification using field samples such as environmental water is also in progress.

【P-64】

環境 DNA で陸の脊椎動物を検出 ～ペイントローラーによる地表面調査の実証

Detecting terrestrial vertebrates using eDNA: A field validation of ground surface sampling with a paint roller

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これまで環境 DNA を使った生物調査は水圏での利用が主であり、水試料から DNA を抽出し、メタバーコーディング法により複数の生物種を効率的に検出できることが報告されている。魚類やほ乳類など分類群ごとに専用プライマーを用いることで、水中で生活する生物だけでなく、水場を利用する陸域生物への適用事例も増えている。一方で、森林や草地といった陸域環境に生息する脊椎動物の調査について、環境 DNA 調査に対する期待は大きいものの、その知見は限られている。そこで本研究では、ペイントローラーを用いた地表面からの DNA 採取方法について、その実施方法について検討を行った。さらに、ほ乳類、鳥類、両生類、爬虫類を対象に、既存の複数のプライマーによる検出種の比較も行った。結果として、ペイントローラーでの環境 DNA の採取は、適切な地表面の状態であれば陸域の脊椎動物の大まかな把握手法として有効であることが示された。

Environmental DNA (eDNA)-based biological surveys are currently mainly applied in aquatic environments. In these settings, DNA is extracted from water samples and analyzed using metabarcoding techniques, enabling efficient detection of multiple species. Specific primers have been developed for various taxonomic groups such as fish and mammals, allowing not only the detection of aquatic organisms but also terrestrial species that utilize water sources.On the other hand, although there is a growing demand for eDNA surveys targeting terrestrial vertebrates including mammals and reptiles, current knowledge in this area remains limited. This study examined a method for collecting eDNA from ground surfaces using a paint roller, focusing on the implementation procedure and the effect of sampling area.Additionally, we compared the species detected using existing primer sets, targeting mammals, birds, amphibians, and reptiles. These results demonstrated that, under appropriate surface conditions, eDNA collection using a paint roller can serve as an effective method for broadly assessing terrestrial vertebrate presence.

【P-65】

環境 DNA を用いた里山における野生生物モニタリング手法の検討

Evaluation of wildlife monitoring approaches in Satoyama landscapes through environmental DNA

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福岡県太宰府市の里山において、環境 DNA を用いた野生生物モニタリング手法を検討した。本研究では陸生哺乳類を対象とし、自動撮影カメラおよび超音波レコーダーを組み合わせて生息状況を調査するとともに、DNA 検出に適した環境媒体を探索した。落葉や低木の洗浄水、また自動撮影カメラで接触が確認された岩石や樹木の拭き取り綿から DNA を抽出した。その結果、撮影頻度の高い種は複数の媒体で高い検出率を示した。さらに、設置期間を変えた調査地の下流における水系のパッシブサンプリングでは、2 週間で最多となる 7 種の陸生哺乳類を検出した。また、超音波解析では複数種のコウモリの存在が確認されたが、本研究で検討した媒体から DNA は検出されなかった。本発表では、夏季・秋季・冬季の調査結果や、パッシブサンプリングの設置期間による違いについても報告する。本研究の一部は、JSPS 科研費 JP25K01361 の助成を受けて実施した。

We investigated wildlife monitoring methods using environmental DNA in the satoyama landscapes of Dazaifu City, Fukuoka Prefecture, Japan. This study focused on terrestrial mammals, combining camera traps and ultrasonic recorder to survey their occurrence while also exploring suitable environmental media for DNA detection. DNA was extracted from wash water of leaf litter and shrubs, as well as from swabs of rocks and trees where contact was captured by camera traps. The results showed that species with higher photographic capture frequencies exhibited higher detection rates across multiple media. Furthermore, passive water sampling conducted downstream of survey sites with different installation periods detected up to seven terrestrial mammal species during a two-week deployment, representing the highest yield among tested durations. Ultrasound analysis confirmed the presence of multiple bat species; however, their DNA was not detected in the environmental media examined in this study. In this presentation, we will also report seasonal results from summer, autumn, and winter, as well as differences associated with passive sampling durations.Part of this research was supported by JSPS KAKENHI Grant Number JP25K01361.

【P-66】

埼玉県における野生イノシシ検知を目的としたアジア型ミトコンドリア DNA を標的とする qPCR 検出系の開発

Development of a qPCR assay targeting Asian-haplotype mtDNA to detect wild boar (*Sus scrofa*) in Saitama

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野生イノシシによる農作物被害は深刻であり、近年では養豚における豚熱感染リスクの要因とも考えられている。そのため、捕獲の強化などの対策が進められている。効果的に対策を進めるためには野生イノシシの生息範囲や密度を把握する必要があり、従来の調査手法を代替または補完する技術として環境 DNA 調査に注目が集まっている。そこで、環境試料から野生イノシシを特異的に検知することを目的としてリアルタイム PCR（qPCR）検出系を開発した。特異的プライマーとプローブの設計にあたって、アジア型のミトコンドリア DNA 塩基配列を標的とした。設計したプライマー・プローブの特異性は、埼玉県内の野生イノシシおよびその他哺乳類の DNA を用いて検証した。また、人工的にイノシシの血清を添加した環境水および土壌から DNA を抽出し、特異性が確認されたプライマー・プローブを用いてイノシシ DNA が検知可能であることを確認した。

Wild boars are problematic due to the damage they cause to crops, as well as their potential to transmit classical swine fever to domestic pigs. Therefore, measures to capture them have been strengthened in recent years. In order to implement these measures effectively, it is important to understand the size and density of wild boar populations. Environmental DNA (eDNA) surveys are attracting attention as a potential replacement or supplement to conventional survey methods. This study developed a real-time PCR (qPCR) detection system specifically targeting wild boars in environmental samples.
Specific primers and probes were designed using the mitochondrial DNA sequence of the Asian-haplotype. The specificity of the primers and probes was verified using DNA from wild boars and other mammals in Saitama Prefecture. Furthermore, DNA was extracted from water and soil samples that had been artificially spiked with wild boar serum. Using the designed primers and probes, we confirmed that wild boar DNA could be detected in these samples.

【P-67】

Seasonal diet of wild boar (*Sus scrofa*) revealed by DNA metabarcoding analysis

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After the Fukushima Daiichi nuclear power plant accident, radioactive cesium concentration in wild boar (*Sus scrofa*) meat tends to be high, and seasonal fluctuations have been detected. In addition, radioactive concentration in wild boar muscles showed a positive correlation with those in the stomach contents of the same individuals. Therefore, diet may be an important factor in understanding cesium uptake in wild boars. In this study, we conducted a dietary analysis using metabarcoding to reveal the seasonal diets of wild boars in Nihonmatsu City, Fukushima Prefecture. We analyzed DNA sequences of the ITS-2 region to identify plant species in the stomach contents of 174 wild boar samples. In total, 60 families and 172 species were detected. A variety of hard mast species, such as oak and chestnut, were frequently detected from autumn to winter. In winter, plants such as fish mint and dandelion were also consumed. From spring to summer, plant species from the Japanese parsley and cherry (*Prunus* spp.) were detected. These results indicate that wild boars utilize a wide range of plant resources as diet throughout the year.

【P-68】
パッシブサンプリング法による河川での魚類環境 DNA 調査への適用に関する検証

Evaluation of the application for fish environmental DNA survey in rivers using the passive sampling method

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近年、新たな環境 DNA 調査の手法として、環境 DNA の捕集材を環境中に設置し、一定期間後に回収するパッシブサンプリング法（PS 法）が提案されている。PS 法は捕集材を長時間浸漬することで生物情報を時間積算的に蓄積し、夜行性の種や感潮域の種を省力的かつ効率的に検出することが期待できる。本研究では、PS 法における種の検出率向上に関する検討を目的として、環境の異なる複数の河川において PS 法を用いた魚類環境 DNA 調査を実施した。その結果、PS 法は従来の採水法や採捕調査よりも多くの魚種を検出し、①夜行性の魚種や重要種等の出現頻度が少ない種を検出する場合、②感潮域のような時間変化が大きい環境で調査する場合に、特に効果的であることが示唆された。さらに感潮域では、捕集材に付着した沈着物も並行して分析することで魚類の検出率が向上することが明らかとなった。

In recent years, the passive sampling (PS) method has been proposed as a novel tool for environmental DNA (eDNA) surveys. The PS method accumulates eDNA by immersing collection materials for a long period, enabling the efficient and labor-saving detection of nocturnal and tidal-zone species. In this study, fish eDNA surveys using the PS method were conducted in multiple rivers with diverse environments to examine the improvement of species detection in the PS method. As a result, the PS method detected more fish species than water sampling and capturing methods. The PS method was also particularly useful for: ① detecting species with low occurrence, such as nocturnal or rare species, and ② surveying environments with large temporal variations, such as the tidal zone. Furthermore, in the tidal zone, fish detection rate was much improved by simultaneously analyzing the sediments covering the collection materials.

【P-69】
環境 DNA パッシブサンプリング法による遠賀川水系の魚類相把握

Surveying the fish fauna of the Onga River system using an environmental DNA passive sampling method

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環境 DNA の標準的な調査方法である採水法は、瞬間値を捉える特性上、自然環境下における種の検出率や網羅性に課題があった。近年、海綿で DNA を長時間捕集するパッシブサンプリング法 (PS 法) が開発され、上記課題の解決が期待されている。本研究では、河川水辺の国勢調査等で経年的に魚類調査が実施されている遠賀川水系において PS 法を実施し、既往調査結果と比較した。河川水辺の国勢調査における調査地区やワンド等 21 地区で PS 法を実施し、71 検体を魚類環境 DNA メタバーコーディングに供した。PS 法で既往確認種の 42%、採捕での確認が難しい重要種を複数検出し、ワンド等の環境区分により検出種が異なった。また、一部地区では採水法も実施したところ、感潮域では PS 法より多くの種が検出された。PS 法は個体数が少なく採捕が難しい種の検出に有効であるが、潮汐によりトラップされた泥成分等が分析に悪影響を与えることが示唆された。

Water sampling, one of the standard method of environmental DNA (eDNA) analyses, has limitations in the detection rate and comprehensiveness of species diversity in natural environments due to the snapshot information. Recently, passive eDNA sampling (PS) using sponge skeletons is a novel alternative to water sampling, and is expected to overcome these challenges. In this study, we conducted the PS method and water sampling in the Onga River system and compared our results with previous survey data of long-term fish surveys in National Survey On Natural Environment In River And Watershore. Total of 71 PS samples were collected from 21 sites including census survey areas and side channels (wando), and were subjected to fish eDNA metabarcoding. The PS method successfully detected 42% of previously observed fish species, including various important species that are difficult to capture by conventional method such as net-capturing. Fish species compositions are different between environmental classification such as river side pool (Wando). On the otherhand, water sampling had higher species diversity than PS method in the tidal zone. These results suggest that PS method is effective for detecting low-abundance species, but sediment components on PS material could negatively affect the efficiency of eDNA extraction and DNA amplification.

【P-70】

降雨時河川水の環境 DNA 分析による集水域の哺乳類・鳥類相評価法の検討：採水法とパッシブサンプリング法の比較

Evaluation of mammal and bird communities in a catchment using environmental DNA from rainfall-runoff river water: Comparison of water and passive sampling methods

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降雨時の河川水は河畔域から流出した環境 DNA を含み，流域全体の生物多様性を反映する可能性がある．本研究では，降雨時河川水の環境 DNA 分析により，集水域に生息する哺乳類・鳥類相を概括的に把握する手法の提案を目的とした．中国地方の一級河川である佐波川の中流域 1 地点において，降雨開始からピークを経てその後に至るまで 2 時間ごとに採水し，環境 DNA メタバーコーディングにより哺乳類・鳥類相を推定した．さらに，乾燥海綿を捕集材としたパッシブサンプリング法（PS 法）も併用し，採水法と比較した．その結果，哺乳類 28 種，鳥類 19 種が検出され，PS 法（哺乳類 9 ～ 22 種、鳥類 6 ～ 11 種）が採水法（哺乳類 4 ～ 15 種，鳥類 1 ～ 8 種）より多くなる傾向を示した．哺乳類の 64%，鳥類の 63% が両手法で共通して出現し，PS 法は採水法の大部分の種を網羅したことから，哺乳類・鳥類相をより効率的に把握できることが示唆された．

Rainfall-runoff river water contains environmental DNA (eDNA) originating from riparian zones and may reflect overall watershed biodiversity. This study aimed to propose an approach for comprehensively assessing mammal and bird communities within a catchment using eDNA analysis of river water during rainfall events. At a midstream site of the Saba River, a first-class river in the Chugoku region of Japan, water samples were collected every two hours from the onset of rainfall through the peak and subsequent period. Mammal and bird communities were inferred via metabarcoding. Simultaneously, passive sampling was conducted using dried sponges, and results were compared with those from water sampling. Overall, 28 mammal species and 19 bird species were detected. The number of species tended to be higher in passive sampling (mammals: 9–22; birds: 6–11) than in water sampling (mammals: 4–15; birds: 1–8). Sixty-four percent of mammal species and 63% of bird species were detected by both methods, and passive sampling captured most species found by water sampling. These results suggest that passive sampling is an efficient method for assessing mammal and bird communities using eDNA in rainfall-runoff rivers.

【P-71】
パッシブエアサンプラーによる哺乳類、鳥類、植物の調査

Survey of mammals, birds, and plants using passive air samplers

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環境 DNA を用いた生物調査は、様々な場面で使用されるようになってきたが、ほとんどは水を対象としたサンプリングが実施されている。近年では、空気や土壌を対象とした環境 DNA のサンプリングも行われつつあるが、発展途上であり効果的な調査手法は確立されていない。空気中の環境 DNA をサンプリングする際には、アクティブサンプラーが用いられることが多いが、電力を使用するため野外での設置に労力がかかる、長時間の運用が難しいため設置時の気象条件に調査結果が大きく左右されるといった問題がある。本研究では、電力を使わず長時間設置できるパッシブエアサンプラーを用いて空気中の環境 DNA をサンプリングし、滋賀県東近江市の林内において哺乳類、鳥類、植物を対象とした調査を実施した。さらに、IC レコーダーやセンサーカメラによる調査、植物相調査等も同時に実施し、パッシブエアサンプラーによる環境 DNA 調査の結果と比較した。

Environmental DNA (eDNA) surveys have increasingly been applied in various contexts, but most studies so far have focused on sampling from water. In recent years, attempts have also been made to collect eDNA from air and soil; however, these approaches are still in the developmental stage, and effective survey methods have yet to be established. When sampling airborne eDNA, active samplers such as air pumps are often used, but their reliance on electricity poses challenges: they require considerable effort for field deployment, long-term operation is difficult, and results can be greatly influenced by weather conditions at the time of sampling.In this study, we conducted surveys targeting mammals, birds, and plants in a forest in Higashiomi City, Shiga Prefecture, using passive air samplers, which require no power supply and can be deployed for extended periods. In addition, surveys using IC recorders and sensor cameras, and a floral survey were carried out in parallel, and the results were compared with those obtained from the passive air sampler eDNA surveys.

【P-72】

大気エアロゾル試料を用いた環境 DNA メタバーコーディングによる生物モニタリングの可能性

Potential of environmental DNA metabarcoding using aerosol samples for biodiversity monitoring

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バイオエアロゾルとは、大気中を浮遊する粒子状物質のうち、生物に由来する物質の総称である。これまで花粉、有機化合物、微生物などが主な対象であったが、近年、そこに動植物由来 DNA が含まれることが明らかとなり、環境 DNA 分析による生物モニタリングを可能とする試料として大きな注目を集めている。本研究では、有害大気汚染物質モニタリングのために採取されたエアロゾル試料を用い、環境 DNA 分析による陸上生物の検出を試みたので、その結果について報告する。

Bioaerosols are defined as airborne particulate matter of biological origin. Traditionally, research has mainly focused on pollen, organic compounds, and microorganisms contained within them. More recently, bioaerosols have attracted growing interest for eDNA-based monitoring, as it has been demonstrated that they also harbor DNA derived from plants and animals. In this study, we analyzed aerosol samples collected for hazardous air pollutant monitoring to detect terrestrial organisms, and here we report our findings.

【P-73】

Methodological assessment of rotorod-based airborne eDNA collection in a cave environment

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Airborne environmental DNA (eDNA) is the DNA shed by organisms into the environment that becomes suspended in the air as particles or aerosols, and can be collected and analyzed to detect the presence of species. It is particularly effective in semi-open environments where air flows are more constrained and DNA molecules may accumulate. To evaluate its potential applications in monitoring cave ecosystem, we compared two adhesion methods of rotorod sampler for airborne eDNA collection and assessed potential temporal lag effects of eDNA signals. We established a sample station within a cave known to be utilized by the great roundleaf bat *Hipposideros armiger* in northeastern Taiwan. A rotorod sampler with two rotating arms was fitted with cotton swabs coated with either Vaseline or attached with a tape. Continuous weekly sampling was carried out from September to October in 2025. Infrared-triggered camera traps were also deployed to record other animals' access to the cave. Collected eDNA samples will undergo sequencing and meta-barcoding for species identification, with results compared against camera-trap footage. As the bats are expected to migrate away by mid-fall (mostly in October), which allowed us to compare the samples pre- and post-migration to investigate the temporal persistence of airborne eDNA. This study is expected to provide methodological validation for the use of rotorod sampler in airborne eDNA sampling in subtropical semi-open environments.

【P-74】

Exploring the potential of airborne environmental DNA for terrestrial wildlife monitoring

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Airborne environmental DNA (eDNA) analysis is a developing research field worldwide, and knowledge regarding the relationship between air sampling volume and detection yield, as well as detection range, remains limited. In this study, we conducted experiments on airborne eDNA targeting the Japanese squirrel (*Sciurus lis*), a species listed as Endangered Category I in Hiroshima Prefecture, at Fukuyama City Zoo. The aim was to clarify the basic characteristics of airborne eDNA and to evaluate its potential application to biomonitoring surveys. Air was collected onto filters using a custom-built air sampler at the Zoo, after which DNA was extracted from the filters. The obtained airborne eDNA samples were analyzed using species-specific PCR primers for the *S. lis* through quantitative PCR, and metabarcoding analysis was also performed to assess the detectable range. As a result, Japanese squirrel DNA was successfully detected in air samples collected in front of outdoor enclosures, whereas detection at sites approximately 30 m away was more strongly influenced by weather conditions. This study provides preliminary insights into the applicability of airborne eDNA techniques for monitoring terrestrial mammals.

【P-75】

Airborne eDNA metabarcoding reveals locatio-specific zoo mammal faunas

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Recent progress in airborne environmental DNA (eDNAir) techniques is enabling more cost-effective approaches to biodiversity monitoring. Although previous studies have reported that atmospheric environmental factors and animal biomass affect detection sensitivity, the physicochemical dynamics of eDNAir remain largely elusive. To our knowledge, information regarding the extent to which eDNAir reflects the physical range of fauna is particularly lacking. Therefore, this study hypothesized that animal communities detected at two locations within Hiroshima City Asa Zoological Park (Asa Zoo) might differ. Consequently, we collected 18,000 ℓ of air sampling (about 2 hours) by using 3D-printed devices at 2 different sites in the zoo. Then, we extracted eDNA and conducted PCR and metabarcoding. For raw read analysis, we performed BLAST-based taxonomy assignment using the qiime2 pipeline. Diversity analysis was performed on the data obtained from these operations, visualizing the differences between locations. The results revealed differences in the biota detected at the two sites. The species composition at each site revealed that a large proportion of the animals detected were those kept nearby, with the straight distance between the two sites being approximately 400 meters. Under the conditions of this study, it was inferred that eDNAir reflects a regional wildlife community. This finding is expected to be useful for identifying the habitats of endangered small mammals and birds that are difficult to survey. Future work will integrate meteorological factors and create a material transport model with the aim of extending applications beyond the zoo to field sites.

【P-76】
室内の空気を用いたヒト環境 DNA 解析の法科学的応用

Forensic applications of human environmental DNA analysis using indoor air

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事件や事故の現場における人の行動を把握することは、犯罪捜査を行う上で重要である。我々は、室内の空気中に存在するヒト環境 DNA（eDNA）に着目し、eDNA を分析することで、その部屋にいた人物を特定するとともにその行動を推測することが可能か否かを検討したので報告する。複数の人物が使用する部屋を研究対象とし、室内空気中の eDNA の採集には HEPA フィルターを吸引口に装着したハンディ掃除機を使用した。空室状態で採集した室内空気中の eDNA からヒト DNA マーカーは殆ど検出されなかったが、室内に被験者がいる状態で採集した eDNA からは被験者の有する DNA プロファイルに対応する DNA マーカーが明瞭に検出された。以上の結果から、室内空気中の eDNA 解析により、eDNA を採集した時点でその場にいた人物を特定でき、その人物の行動も推測可能であると考えられた。

Understanding human behavior at crime scenes or accident sites is crucial for conducting criminal investigations. We focused on human environmental DNA (eDNA) present in indoor air and explored whether it is possible to identify individuals who were in a room and infer their behavior by analyzing their DNA. This study was conducted in a room used by multiple people. A handheld vacuum cleaner fitted with a HEPA filter on its suction inlet was used to collect eDNA from the indoor air. When the room was empty, human DNA markers were scarcely detected in eDNA from the indoor air. However, when participants were present in the room, the DNA markers corresponding to their DNA profiles were clearly detected. These results suggest that analyzing eDNA in indoor air could identify individuals present at the time of eDNA collection and potentially infer their activities.

【P-77】
環境 DNA 分析におけるガラスファイバーフィルターの性能比較

Performance comparison of glass fiber filters in environmental DNA (eDNA) analysis

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環境 DNA（eDNA）分析におけるろ過工程では、「環境 DNA 調査・実験マニュアル」に基づき、①ガラスファイバー製、②孔径が 0.7 μm 程度の条件を満たすろ紙として Whatman GF/F フィルター（ガラスファイバー製・孔径 0.7 μm ）（GE Healthcare 社）が使用されることが多い。一方で、同等の性能を持つフィルターは他社からも多数市販されているが、メーカーや製品による違いを比較した研究は少ない。本研究では複数のフィルターを選定し、GF/F との比較を行った。評価は、魚類の網羅的解析における検出種数および種組成の比較に加え、各フィルターの再現性（同一条件下での検出種・組成の安定性）、作業性（ろ過に要する時間）を指標とした。その結果、フィルターごとに特性があることが明らかとなった。本発表では、各フィルターの特性と eDNA 分析への適用可能性について報告する。

In environmental DNA (eDNA) analysis, the filtration process often follows the *Environmental DNA Sampling and Experiment Manual* by the eDNA Society, which recommends using filter papers that meet the following criteria: (1) made of glass fiber, and (2) with a pore size of approximately 0.7 μm. Accordingly, the Whatman GF/F filter (glass fiber, 0.7 μm pore size) () is commonly used. Meanwhile, although many other manufacturers offer filters with similar specifications, few studies have compared the differences among filters of the same pore size and material (glass fiber) from different brands and products. In this study, we selected several filters and compared them with the commonly used GF/F filter. The comparison focused on three criteria: the number of detected species and species composition in MiFish metabarcoding analysis, the reproducibility of each filter (consistency under identical conditions), and operational efficiency, measured by the time required for filtration. The results revealed that each filter has distinct characteristics. In this poster, we report on the properties of each filter and their applicability to eDNA analysis.

【P-78】
環境 DNA 解析より高感度な環境 RNA 解析の比較検証

Comparative verification of highly sensitive environmental RNA analysis than environmental DNA analysis

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魚類の調査では環境 DNA を用いた解析が主流であるが、近年、環境 RNA の研究が進められ、環境 DNA 解析よりも検出感度が優れている可能性が示唆された。しかしながら、両者の量的な比較研究は発展途上である。本研究では、環境 RNA は環境 DNA よりも多く存在するという仮説の下、メダカ水槽や錦鯉が生息する池の水サンプルを対象に定量 PCR を行い、環境 DNA と環境 RNA の検出量を比較した。また、メダカ水槽の実験では、両者の放出速度の比較も試みた。その結果、環境 RNA の検出量が環境 DNA より水槽環境では 8 から 18 倍、池では 13.4 倍ほど多く、また放出速度も環境 RNA の方が速い傾向にあることが分かった。

Environmental DNA (eDNA) analysis is one of the popular methods in fish habitation analysis. Recently, environmental RNA (eRNA) has proposed to improve the sensitivity of the analysis. However, the research of quantitative comparison of those analyses is still insufficient. Based on the hypothesis that eRNA is more abundant than eDNA, we performed quantitative PCR on water samples from medaka aquaria and koi ponds to compare the detectability. In addition, water samples were collected periodically from the medaka aquaria to compare the release rates of eDNA and eRNA. The research indicates that the amount of eRNA was approximately 8 to 18 times higher than that of eDNA in the aquaria environment, and approximately 13.4 times higher in the pond environment. It is also shown that the release rate of eRNA was faster than that of eDNA.

【P-79】
PCR フリーロングリードシーケンシングによる eDNA からのミトコンドリア全長回収

Complete fish mitogenomes from aquatic eDNA using PCR-free long-read sequencing

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eDNA は非侵襲的な生物多様性調査に広く用いられているが、分解を前提に短いバーコード領域に依存してきた。一方、条件次第では mtDNA 全長などの長鎖断片が残存し得ることから、eDNA から mtDNA 全長配列を取得するロングリード・ワークフローを構築した。キンギョとメダカの飼育水を用いてろ過・抽出条件を最適化し、PCR フリーとロングレンジ PCR の二戦略を検証した。PCR フリーでも 16 kb 超リードを多数取得し、mtDNA 全長の *de novo* アセンブリが可能であった。PCR 適用時は mtDNA 由来リードの選別とサブサンプリングでアセンブリが堅牢化した。さらに、単独飼育水由来のアセンブリ配列の比較では、mtDNA 全長上の 2 座位で配列が二分された。本手法は養殖や保全などの管理環境における非侵襲的な高解像度モニタリングに有望であり、野外適用に向けてもサンプリングや前処理の工夫次第で大きな展開が期待される。

Environmental DNA (eDNA) enables noninvasive biodiversity surveys but is usually treated as highly fragmented, so most work has targeted short mitochondrial barcodes. We asked whether intact or near-intact mitogenomes persist in water and developed a long-read workflow to recover them directly from eDNA. In aquaria with *Carassius auratus* and *Oryzias latipes*, we optimized filtration and extraction for high-molecular-weight DNA and evaluated two nanopore strategies: PCR-free long-read sequencing of native eDNA and long-range PCR followed by long-read sequencing.Under the optimized conditions, PCR-free datasets from single-fish tanks yielded up to ~800 mitochondrial reads, including > 16-kb molecules that enabled *de novo* assembly of complete mitogenomes. When starting mtDNA was low, long-range PCR was effective; after amplification, nanopore sequencing provided ~10^5x mitogenome depth, and robust assemblies followed with minimal preprocessing (selecting mtDNA-mapped or 16-18 kb reads and applying light subsampling). Assembled sequences exactly matched the tissue-derived consensus and were at least 99.9% identical to public reference assemblies, supporting accuracy. Across single-fish tanks, alignment of assemblies identified two SNVs (a D-loop substitution and a single-base deletion in 16S rRNA) that separated samples. Read-level evidence confirmed these calls, and in one water sample long reads spanning both sites carried both haplotypes, suggesting possible heteroplasmy. Together, these results show that complete fish mitogenomes can be reconstructed directly from aquatic eDNA, moving eDNA beyond species detection toward haplotype-scale genetics. The approach is practical for managed systems (e. g., aquaculture, seed-production/hatchery, conservation facilities) and provides a path to applications in natural waters with appropriate adaptations in sampling and preprocessing.

【P-80】

A novel octocoral-specific primer of the mitochondrial MutS-like protein (mtMutS) gene for environmental DNA (eDNA) metabarcoding

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The taxonomy of octocorals (class Octocorallia, phylum Anthozoa) faces systematic challenges, yet their diversity and their ecological roles remain poorly understood. Along with traditional surveys, environmental DNA (eDNA) offers a promising non-invasive, rapid, and cost-effective tool for monitoring coral reef communities, and for tracking changes due to anthropogenic pressures and climate change. Most anthozoan eDNA studies have focused on the class Hexacorallia, while studies on Octocorallia remain limited. Octocoral primers thus far were developed either specifically for deep-sea octocorals (mitochondrial MutS) or for all anthozoans (e.g. nuclear 28S rRNA). In this study, we developed a new octocoral-specific primer set targeting the mitochondrial MutS gene for environmental DNA (eDNA) metabarcoding and compared its performance with the two previous commonly-used primer sets. In this study, photo-transect surveys were conducted, and seawater samples were collected at shallow coral reefs in Okinawa, Japan. Samples were amplified using the nuclear gene and two primer sets of the mitochondrial gene. Via detection of octocoral ASVs from seawater, 28S rRNA obtained seven families from two orders, MutS revealed 12 families from one order, and the new primer set identified 10 families from two orders. All three primer sets detected Nephtheidae, Sarcophytidae, Sinulariidae, and Xeniidae. We recommend sequencing multiple markers or optimization with multiplexing to obtain higher octocoral metabarcoding coverage. Such biodiversity studies testing the performance of eDNA primers sets are important to confirm the best approaches for biodiversity monitoring, to understand how coral reefs will continue to change, and to implement management strategies to ensure their future.

【P-81】
高効率 eDNA 濃縮手法 QuickConc の大容量試料への適用

Application of the high-efficiency eDNA concentration method, QuickConc, to large-volume samples

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環境 DNA（eDNA）分析は、非侵襲的な生態モニタリング手法として注目されている。筆者らはこれまでに迅速かつ電力不要で現場濃縮が可能な QuickConc 法を開発したが、大容量の試料処理が困難という課題があった。この課題を克服するため、吸引ろ過を組み合わせた QuickConc Vacuum 法を新たに開発した。本研究では、水族館と神戸港で採水した試料を用い、新手法と既存のガラス膜や Sterivex 法とで種特異的 eDNA 量（qPCR）およびメタバーコーディングによる検出魚種数を比較した。いずれの手法も試料量に相関して eDNA の収量が向上し、特に 2L の海水試料において、QuickConc Vacuum 法は既存手法よりも有意に多くの魚種を検出した。QuickConc Vacuum 法は従来の課題であった処理可能なサンプル量の限界を克服し、生物多様性モニタリングの新たなツールとしての活用が期待される。

Environmental DNA (eDNA) analysis is a notable non-invasive method for ecological monitoring. To address challenges like sample transport and handling, we previously developed QuickConc method, a rapid, power-free, on-site concentration technique using dispersed glass fiber sheets and cationic surfactants. However, this manual method was unsuitable for large-volume samples. To overcome this limitation, a new method called QuickConc Vacuum was developed by combining the original technique with vacuum filtration. This study evaluated its effectiveness from aquarium and seawater samples ranged from 0.5L to 5L against conventional glass membrane and Sterivex methods, using metabarcoding and qPCR. The results showed that increasing the sample volume resulted in a higher eDNA concentration with a significant positive correlation. In metabarcoding analysis, QuickConc Vacuum demonstrated a significantly higher number of detected fish species, particularly in 2L seawater samples. This study concludes that the QuickConc Vacuum method successfully overcomes the volume limitations of its previous method. It shows higher eDNA recovery and species detection compared to existing methods, positioning it as a promising new tool for biodiversity monitoring.

【P-82】

濁水条件下の環境 DNA 回収における新規現地濃縮法 QuickConc の有用性評価

Evaluation of QuickConc, a novel on-site concentration method, for environmental DNA recovery under turbid water conditions

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環境 DNA（eDNA）の検出精度は生物・環境的な条件に左右されるが，濁水中の eDNA の動態に関する知見は限られている．特に，現地ろ過・濃縮手法による濁水処理性能については全く知見がないのが現状である．本研究では，濁水条件下における新規濃縮法 QuickConc と従来濃縮法による eDNA 回収効率を比較した．対象とする濁水試料は，コイを飼育する水槽水と 2 種類の鉱物（カオリン，モンモリロナイト）を混合し，目標濁度を 0 ～ 200 ppm（5 通り）に設定して各 2 反復作成した．試料水作成後，ガラス繊維ろ紙 GF/F と QuickConc を用いて eDNA を濃縮し，DNA を抽出した．コイの eDNA 濃度の定量には，デジタル PCR を使用した．結果として，両鉱物に共通して，GF/F では中～高濁度域に eDNA が検出できなかった一方，QuickConc では GF/F よりも eDNA 濃度が高くなる傾向が示された．

The detection accuracy of environmental DNA (eDNA) depends on biological and environmental factors, but knowledge of eDNA dynamics in turbid water is limited. In this study, we compared the recovery efficiency of a novel concentration method, QuickConc, with a conventional method under turbid conditions. Turbid samples were prepared by mixing carp tank water with two minerals (kaolin and montmorillonite) at five target turbidity levels (0–200 ppm) in duplicate. After concentration using GF/F filters or QuickConc, DNA was extracted. Subsequently, the carp eDNA concentration was quantified by digital PCR. QuickConc tended to yield higher eDNA concentrations than GF/F, which failed to detect eDNA at medium to high turbidity.

【P-83】
ハイブリダイゼーション MIG-seq（hyMIG-seq）法による環境 DNA 分析

Hybridization MIG-seq (hyMIG-seq) for the environmental DNA analysis

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ターゲットキャプチャー法は、ビオチン結合させた標的配列プローブと磁気ビーズを用いて、標的配列を選択的に濃縮する方法である。本研究では、ゲノムワイド SNP 分析法の一つである MIG-seq 法にこの手法を応用して（hyMIG-seq 法）、土壌試料の環境 DNA 分析を実施した。まずブナの葉から抽出した DNA を用いてプローブを作成し、ブナが優占する森林土壌から抽出した DNA を対象にターゲットキャプチャーを行った。その結果、MIG-seq 法で直接森林土壌の DNA を分析した場合と比較し、hyMIG-seq 法では最大 100 倍程度のゲノムワイド SNP を取得できた。次にこの技術を用いて、縄文時代の遺跡から得られた古代土壌試料の分析を実施した。その結果、当時道具として使用されていたと考えられるタケ類や、食料とされていたイノシシをプローブにすることで、現生個体との遺伝的多様性比較や系統関係を把握することに成功した。

The target capture method uses biotin-combined target sequence probes and magnetic beads to selectively enrich a portion of the target species' genomic regions in extracted DNA. We applied this technique to a genome-wide SNP analysis, the MIG-seq method. For the first experiment, we created target sequence probes from DNA extracted from the leaves of a tree species (beech; *Fagus crenata*). Using the probes, we employed the target capture method on environmental DNA extracted from forest soil collected in a beech-dominated forest. As a result of this study, we can obtain over one hundred times more SNP loci compared with those using the usual MIG-seq method from the same sample. For the second experiment, ancient soil samples from the Jomon era were utilized with the hyMIG-seq method. As a result, we successfully measured genetic diversity and revealed phylogenetic relationships of bamboo species and wild boars which were used for ancient tools and food sources. This technology is expected to expand the possibilities of environmental DNA analysis significantly.

【P-84】
PMiFish ver.3.0：環境 DNA メタバーコーディング解析パイプラインの改良

PMiFish ver.3.0: An improved pipeline for eDNA metabarcoding workflows

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環境 DNA メタバーコーディング解析は、次世代シーケンサーのリード処理に専門知識を要し、初心者には難しい。そこで著者は、Illumina シーケンサー出力から分類群へのアサインメントまで自動処理できる解析パイプライン PMiFish ver.2.4 を開発した。当初は MiFish プライマー用だったが、任意の分類群に対応可能で、複数プライマー使用時もそれぞれ結果を出力できる点が特徴である。最新版の PMiFish ver.3.0 では、USEARCH に加え VSEARCH を利用でき、Windows 環境ではデノイズに DADA2 も選択可能となった。さらに主要プログラムのオプション設定の柔軟化や demultiplex 機能が追加され、環境 DNA 研究者がより快適に解析を実施できる環境を提供する。

Environmental DNA (eDNA) metabarcoding has become a widely used approach for biodiversity monitoring, but processing sequencing reads from next-generation sequencers often requires specialized bioinformatics skills and can be challenging for beginners. To facilitate this process, I previously developed PMiFish ver.2.4, a pipeline that automates the workflow from Illumina fastq output to taxonomic assignment. Although originally designed for MiFish primers, PMiFish can be applied to any taxonomic group, and it has a distinctive feature that allows users to analyze multiple primer sets within a single sample, providing separate results for each primer. In this presentation, I introduce PMiFish ver.3.0, a major update with several important improvements. First, in addition to USEARCH, the pipeline now supports VSEARCH, providing users with a free and open-source alternative. Second, for Windows environments, DADA2 can be employed to perform denoising-based analysis. Third, a demultiplexing function has been added, enabling flexible handling of multiplexed datasets. Finally, the new version offers greater flexibility in setting options for USEARCH, VSEARCH, and DADA2, allowing users to fine-tune analyses according to their specific needs. These updates enhance the versatility, accessibility, and usability of PMiFish, making it a more powerful tool for researchers working with eDNA metabarcoding data. By lowering the technical barrier to analysis, PMiFish ver.3.0 contributes to broader adoption of eDNA methods and to more efficient biodiversity assessments.

【P-85】

Multidimensional community data analysis: Tensor decomposition of spatiotemporal environmental DNA data

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Community monitoring data are increasingly available and often high-dimensional. They include not only multiple variables within a single "category" (e.g., different "dates" in a "time" category) but also multiple variable categories (e.g., "time," "space," "species," etc.). Data with multiple variable categories consist of a three- or higher-dimensional data structure called a "tensor." Dimension reduction techniques, such as Principal Component Analysis (PCA), are essential for visualizing overall patterns and extracting essential features for subsequent analysis. However, flattening a tensor into a matrix and applying PCA or other "2-way" analysis methods may misidentify or overlook important features of tensor data. In this study, I applied tensor decomposition, which directly decomposes a tensor into key components, to spatiotemporal environmental DNA (eDNA) monitoring data and compared its performance with traditional 2-way data analysis methods such as PCA. Tensor decomposition more clearly extracts spatial and temporal components of eDNA monitoring data, suggesting it is a potentially useful and more effective method for visualizing and extracting essential features of eDNA monitoring data. Given the increasing temporal frequency and broader spatial coverage of eDNA monitoring data, the development of high-dimensional data analysis methods is critical for more efficient and accurate biodiversity monitoring.

【P-86】

HydroGen: Integration of DNA-based assessment tools into water quality and biodiversity monitoring

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Traditional biodiversity monitoring often faces challenges such as misidentification, low detection probabilities, and reliance on invasive methods. Environmental DNA (eDNA) offers a non-invasive, sensitive, and scalable alternative, enabling the detection of organisms from genetic material shed into water, soil, or air. When coupled with metabarcoding, which applies high-throughput sequencing to target multiple taxa simultaneously, eDNA provides rapid and cost-effective biodiversity assessments with improved taxonomic resolution and reproducibility. HydroGen is a two-year monitoring programme across 40 Irish freshwater and transitional ecosystems, spanning a gradient from pristine to heavily impacted sites. Thirty-six sites are sampled annually, while four intensive sites undergo seasonal sampling, allowing for the assessment of both broad-scale spatial patterns and fine-scale temporal dynamics. The project employs a multi-taxa framework, integrating traditional and molecular approaches. Aquatic invertebrates are examined as bioindicators of ecological condition and for their ecological and economic significance. Microbial communities are also targeted, with microbial source tracking (MST) used to characterise contamination sources and evaluate microbial responses to environmental stressors. Diatoms and fish assemblages are additionally assessed, providing sensitive and integrative indicators of water quality, habitat structure, and long-term ecosystem health. Preliminary results from the first year of monitoring are presented, highlighting biodiversity patterns across sites and demonstrating the potential of eDNA-based approaches for complementing traditional methods. Through this integrated approach, HydroGen aims to advance standardised monitoring protocols that combine eDNA with conventional methods, strengthen national biodiversity databases, and enhance Ireland's capacity for evidence-based conservation and sustainable water management.

【P-87】

環境エクソソーム／環境 sEVs（eExosomes/esEVs)）：水生生物間の新たなコミュニケーションネットワークの運搬体

Environmental exosomes/sEVs (eExosomes/esEVs): Mediators of a novel communication network among aquatic organisms

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水生生物は周囲の環境水を介して生体成分の交換を行う。このやりとりは生態学的相互作用に深い影響を与える。そこで我々は、エクソソームや細胞外小胞（sEVs）が水中に分泌され、生物間においても生命情報の安定した運搬体として機能する可能性を仮説とした。これを検証するため、我々はアコヤガイ（ *Pinctada fucata* ）を用いた。アコヤガイ飼育水槽の海水及び養殖海域の海水から、限外濾過によりエクソソーム /sEV サイズの画分を取得した。取得した小胞画分を顕微鏡観察により解析したところ、エクソソーム /sEV サイズの小胞が豊富に確認された。これらから RNA を抽出し、small RNA のシーケンシングを実施した。その結果、血漿エクソソームと同一のアコヤガイ特異的 piRNA が確認され、エクソソーム /sEV に関連する mRNA も検出された。これはエクソソームが環境へ放出されていることを示唆する結果である。

Aquatic organisms exchange biological components through the surrounding environmental water. This bidirectional exchange profoundly influences ecological interactions. Then, we hypothesized that, in addition to soluble biomolecules, exosomes and small extracellular vesicles (sEVs) are also secreted into aquatic environments. This allows them to serve as stable carriers of biological information not only among intra-organsims, as they do as land organisms, but also among inter-organisms. To examine this, we used the Akoya pearl oyster (*Pinctada fucata*), the internal exosomes for which we had previously examined. We collected exosomes/sEVs-sized components through ultrafiltration from seawater of the pearl oyster tank and the open water of the culture area. The collected components were characterized using microscopy, revealing abundant exosome/sEV-sized vesicles. We extracted RNAs from the components and performed sequencing of small RNAs. We revealed that the pearl oyster-specific piRNAs were identical to those in hemolymph exosomes. We also detected exosomes/sEVs-related mRNA, indicating the active release of exosomes into the environment. This study is the first to demonstrate that aquatic organisms release exosomes/sEVs containing species-specific nucleic acids into the surrounding water. This suggests exosomes/sEVs transport RNAs and contribute to inter-organismal information networks. We propose these extra-individual exosomes/sEVs as “environmental Exosomes/environmental sEVs (eExosomes/esEVs)”. In addition to the functional implication of eExosomes/esEVs, their potential as an additional target for eDNAs/eRNAs analysis is discussed.

【P-88】
環境 DNA 分析残サンプルのアーカイブ化の取り組み

Archiving environmental DNA samples remaining after analysis and their future use

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土木研究所では、河川やダムの魚類相調査に eDNA 調査を実装し、生物調査の効率化・高度化を図ることを目的に、国土交通省らと連携して令和 2 年度以降全国調査を実施してきた。河川水辺の国勢調査や水質調査に合わせた本調査と、土木研究所が独自に採取したサンプルも含めると、令和 7 年 9 月末の時点で採水地点・採水時期が異なる 5,000 件を超えるサンプルの収集に至っている。これらの eDNA サンプルの多くは MiFish 分析後も約 80 μL の残があることから、分析残サンプルを今後の河川管理に資する研究で活用していく取組を進めており、今年度内には土木研究所が保有するサンプルの水系別の採取地点や採水時期等の情報を土木研究所の HP で公開する予定である。河川管理の現場での環境 DNA 活用の広がりとともに、今後も充実していく本アーカイブを、河川環境の保全に結びつけることができるよう、他機関等との積極的な連携も視野に入れている。

To enhance the efficiency and advancement of biological surveys, the Public Works Research Institute (PWRI), in collaboration with the Ministry of Land, Infrastructure, Transport and Tourism, has been conducting nationwide environmental DNA (eDNA) surveys to investigate fish species in rivers and dams. By the end of August 2025, approximately 5,000 samples had been collected from different sampling locations and times, based on the national census on river environment, water quality investigations, and PWRI original surveys. After MiFish analysis, approximately 80 μ L of the eDNA remained from these samples. PWRI plans to utilize this residual eDNA in research supporting future river management. Moreover, it plans to publish the sampling times and locations within the multiple rivers on its website during the current fiscal year. With the expansion of eDNA use in river management practices, we are also seeking active collaboration with other organizations to establish a connection between this growing archive and the conservation of river environments.

【P-89】
自然共生サイト・OECM における環境 DNA の役割と実証

The role and demonstration of environmental DNA in OECMs and nationally certified sustainably managed natural sites

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近年、生物多様性保全に向けた国際的な取り組みとして「30by30」が推進され、日本においても自然共生サイト（Nationally Certified Sustainably Managed Natural Sites）や OECM の登録が進んでいる。これらの登録に際しては対象地の生物多様性を把握する必要があるが、従来の目視・採捕調査には時間・労力・専門性が求められるなど課題が多い。そこで注目されるのが環境 DNA 手法であり、水域のみならず陸域でも簡便かつ網羅的に生物相を把握できる利点を有する。本研究では、けいはんな学研都市内の事業所において陸域環境 DNA 調査を実施し、その結果を自然共生サイト登録に活用した事例を報告する。さらに、従来調査との比較や運用上の留意点を考察するとともに、環境 DNA が 30by30 目標達成に向けて果たす役割について展望する。

Global efforts toward biodiversity conservation, such as the 30by30 target, are being advanced in Japan through the registration of OECMs and Nationally Certified Sustainably Managed Natural Sites. Effective registration requires comprehensive and reliable biodiversity assessment; however, traditional surveys are labor-intensive, time-consuming, and demand specialized expertise. Environmental DNA (eDNA) offers a promising alternative, enabling efficient and broad detection of species in both aquatic and terrestrial environments. In this study, terrestrial eDNA surveys were conducted at a facility in the Keihanna Science City, and the results provided evidence for its registration as a Nationally Certified Sustainably Managed Natural Site. We discuss the advantages and challenges of eDNA compared to conventional methods and emphasize its increasing role in supporting the achievement of the 30by30 goal.

【P-90】
環境 DNA の社会実装での注意点と改善を考える

Considerations and improvements for the social implementation of environmental DNA

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環境ＤＮＡの膨大な生態学的情は、現在、十分に使いこなせていない状況が発生している。技術そのものへの否定につながりかねない誤解について、注意すべき事例をもとに、対処法を考える。これらの多くは、生物相や生息環境の従来法のデータと組み合わせる工夫で回避や改善が可能である。これらはいわずもがなではあるが、現時点で健全な技術の発展のためにも必要と考え、今回の学会で会員の方々と論じていきたい。

The vast ecological potential of environmental DNA is currently underutilised. Drawing on cautionary examples of misunderstandings that could lead to outright rejection of the technology itself, we must consider countermeasures. Many of these issues can be avoided or mitigated by combining environmental DNA data with conventional methods for assessing biota and habitats. While these measures may seem self-evident, we believe they are essential for the sound development of the technology at this stage. We wish to discuss these matters with members at this conference.

【PS-01】
高校の部活動による低コスト環境 DNA 調査手法の構築

Establishing a low-cost environmental DNA survey method using high school clubs

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本研究は、専門機器をなるべく用いずに高校の部活動レベルでも実施可能な環境 DNA 調査手法を構築し、実際の野外調査および解析を通してその実効性を検証したものである。従来 eDNA 調査には高価な機材と専門的技術が必要とされていたが、本研究では「遠心分離を用いず、注射器を利用した簡易的な DNA 抽出法」「カラムによる濾過とエタノール沈殿」でコストを大幅に削減しながらも、DNA 抽出の前処理までを部活動レベルで実践可能となった。この方法により、神出鬼没なマミズクラゲの分布調査も実施可能となった。制度的・機器的な制約により規制されていた eDNA 調査が高校の教育現場で、「いる／いない」を調べるという最小限の科学的成果を、自校内の資源と工夫で得られることを実証した。今後は、本手法をもとに他校と連携しマニュアルの共有など、全国の高校が実践できるモデルケースを目指す。

【PS-02】
DNA 保存チューブの違いによる DNA 濃度および DNA 量の経時変化の比較

Comparison of temporal changes in DNA concentration and amount depending on the type of DNA storage tubes

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DNA の長期保存において、DNA 濃度の低下要因の一つとして、保存チューブ表面への DNA 分子の吸着が一般的に知られており、これを軽減するために低吸着チューブ（DNA LoBind）が市販されている。本研究では、チューブの性能による DNA 保存状態への違いを検証するため、コイが生息する河川水およびコイの組織から抽出した DNA を用いて、DNA LoBind チューブおよび一般チューブ 3810X による長期保存実験 (冷凍－ 20℃以下の条件下で保存) を行い、保存開始日、6 カ月、1 年、2 年、3 年の各時点で DNA 濃度および DNA 量を定量し、経時変化を比較した。その結果、DNA 濃度は 3810X の方がやや高い傾向を示したものの、DNA 量は両チューブ間に有意な差は認められず、3810X は十分な保存性能が確認された。以上より、コスト制約のある学校教育現場においては、3810X も実用的な選択肢となり得る可能性が示された。

【PS-03】

ツマグロヒョウモン幼虫の生存率に及ぼすナメクジの影響の評価：食草の食痕に残されたDNAを手がかりにして

Evaluating the effect of slugs on the survival rate of Indian fritillary (*Argyreus hyperbius*) larvae: eDNA traces from slug feeding marks on the host plants of the larvae

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ツマグロヒョウモンはタテハチョウ科のチョウの一種である。発表者の過去の実験において本種の幼虫にナメクジの食害のある食草（パンジーなど）を与えた時、食害なしのものに比べて幼虫の生存率が低くなる傾向がみられたため、ナメクジの食痕は幼虫に負の影響を及ぼすと仮説を立てた。そこでまずは、野外の食草に残っている食痕がナメクジのものかどうかを明らかにできるようにチャコウラナメクジの環境DNA手法の開発を試みた。その結果、組織から抽出したDNAサンプルではチャコウラナメクジのDNAは検出できたが、人為的に食害させたパンジーからはDNAは非検出だった。現在、食痕付き食草からのDNA抽出法の再検討を行うとともに、食草に付着したDNAの残存期間などを調べる実験準備を進めている。将来的には、自然環境におけるナメクジの食痕に対するツマグロヒョウモン幼虫の忌避行動の有無などを明らかにしたいと考えている。

【PS-04】
環境 DNA 分析を用いた淡路島におけるナガレホトケドジョウの分布調査

Distribution survey of the *Lefua torrentis* on Awaji Island using environmental DNA

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フクドジョウ科の日本固有種であるナガレホトケドジョウ (*Lefua torrentis*) は長らく未記載とされ、2018 年に新種として記載された淡水魚で、環境省レッドリストにおいて絶滅危惧Ⅰ B 類に指定されている。本州及び四国で本種の生息が確認されているが、本州と四国の間に位置する淡路島における分布情報は限定的で、淡路島南部の数地点において採捕及び目視での調査で生息が報告されているものの、北部での確定的な分布情報はほとんどない。本研究では、これまであまり調査の行われていない淡路島北部を含む淡路島全域の 39 河川で、環境 DNA 分析によるナガレホトケドジョウの生息確認調査を行った。その結果、報告のなかった 15 河川においても本種の DNA の陽性反応が確認された。また、陽性反応の確認された 4 河川でナガレホトケドジョウと思われる個体を採捕した。このことから、淡路島全域にナガレホトケドジョウの生息が期待できる。

【PS-05】

種特異的環境 DNA 手法を用いた淀川水系におけるツチフキの生息状況調査に必要な識別プライマーの設計および検証について

Design and validation of species-specific primers for environmental DNA analysis to assess the distribution of *Abbottina rivularis* in the Yodo River system

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2025 年 4 月、有限会社 a 環境研究所の岡田龍也氏との出会いを契機に、同氏より、2022 年 8 月に高槻市の淀川で約 30 年ぶりに再発見されたツチフキ（コイ科カマツカ亜科）を対象種とした、種特異的環境 DNA 手法を用いたツチフキの生息状況調査の提案を受けた。しかし、種特異的環境 DNA 手法を用いたツチフキの生息状況調査は、これまで学校現場で実施された事例はなく、本取り組みは先進的な試みとなる。本調査では、淀川水系におけるツチフキの生息状況の把握を目的として、ツチフキを特異的に検出可能な識別プライマーの設計を行った。さらに、実際にツチフキを飼育しながら飼育水から抽出した DNA サンプルと設計した識別プライマーを用いて、種特異的環境 DNA 手法（PCR 法および電気泳動法）を行い、識別プライマーの有効性を検証した。

【PS-06】

環境 DNA による京都府由良川水系の魚類群集構造を規定する環境要因の検討

Environmental factors shaping fish assemblage structure in the Yura River system, Kyoto, revealed by environmental DNA

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京都府北部を日本海に向けて流れる由良川は、その上流の一部が兵庫県の加古川と河川争奪を起こしたことが知られている。そのため、由良川水系では、下流域は日本海系の淡水魚が、中・上流域は瀬戸内海系の淡水魚が分布するユニークな河川と考えられている。本研究では、2023 年～ 2025 年の 7 月下旬に、由良川水系を網羅する 22 地点と、隣接する加古川上流 3 地点、桂川上流 2 地点で採水・ろ過を行い、魚類汎用 PCR プライマー MiFish-U (Miya et al. 2015) を用いた環境 DNA メタバーコーディング解析を行った。その結果 63 OTU (Operational taxonomic units) の魚類を検出することができ、その中には瀬戸内海系の魚種も含まれていた。DNA コピー数をもとに非計量多次元尺度構成法（nMDS）を用いて魚類群集構造の地理的パターンを調べたところ、調査地点全域で本流と支流の流程に沿った 5 つのクラスターに分けることができた。また、間接傾度分析では、川幅・水深・NO3-N で由良川水系の魚類群集構造を説明できる可能性が示唆された。

【PS-07】

Environmental DNA reveals enhanced vertebrate biodiversity on artificial oyster reefs

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Artificial reefs are increasingly implemented in coastal restoration and fishery enhancement efforts, yet their influence on local biodiversity remains an area of active investigation. This study used environmental DNA (eDNA) metabarcoding to compare vertebrate biodiversity in water samples collected on and off an artificial oyster reef in the Atlantic Highlands, New Jersey. Ten water samples were collected and analyzed for mitochondrial 12S fish DNA, using a multi-species PCR primer set and high-throughput sequencing. Results revealed a higher DNA concentration and species richness in on-reef samples compared to off-reef samples, with a notable presence of fish species known to inhabit mid-Atlantic coastal waters. Detection included economically and ecologically important taxa, such as sea bass and flounder, consistent with prior ecological observations of the area. These findings confirm that eDNA captures real-time biodiversity signatures and that artificial reef structures likely enhance local vertebrate presence. Despite low eDNA concentrations in some samples, the study supports the reliability of eDNA for species detection and habitat comparison. By demonstrating the utility of eDNA to assess the ecological value of artificial reefs, this research contributes to growing evidence supporting the integration of molecular tools into coastal monitoring and marine resource management.

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