Estimating the spawning activities of fish species using environmental DNA

呉, 盧漢

(Degree)
博士（理学）

(Date of Degree)
2023-03-25

(Date of Publication)
2024-03-01

(Resource Type)
doctoral thesis

(Report Number)
甲第8546号

(URL)
https://hdl.handle.net/20.500.14094/0100482294

論 文 内 容 の 要 旨

氏　　名　呉 盧漢
専　　攻　人間環境学専攻
指導教員氏名　源 利文

論文題目（外国語の場合は，その和訳を併記すること。）
Estimating the spawning activities of fish species using environmental DNA
（環境DNAによる魚類繁殖活動の推定）

論文要旨

The world's fish biodiversity is threatened due to overfishing, climate change, invasive alien species, and others. Problems such as reducing species biomass and diversity need to be addressed urgently. Monitoring the spawning activity of fish is essential for the conservation and management of fish because spawning directly affects population reproduction and growth. Information on the exact timing and location of spawning activities can serve as a basis for establishing closed fishing seasons and (or) areas to reduce interference with the spawning activities of rare or valuable fishery species. It can also provide a time reference for controlling and managing invasive species. Spawning activity depends on the combined effects of many factors such as water temperature, food, sunlight, and water level, on aquatic organisms including fish. Traditional survey methods for estimating fish spawning periods include collecting eggs or catching fish and examining the gonads of adults or otoliths of fries. However, these techniques are time consuming and labor intensive, and they also cause injury in fish and hinder their natural spawning activity. Monitoring the spawning activities of aquatic organisms using noninvasive methods remains a challenge.

Environmental DNA (eDNA) analysis has received attention as a noninvasive survey method. As this method relies on collecting DNA fragments in the water for analysis, such that it can investigate biological population structures in a body of water without damaging the organisms and with a significantly reduced expenditure of time and labor. During spawning, fish release large amounts of

sperm and eggs into the water, which has been assumed to cause an increase in eDNA levels and nuclear eDNA/mitochondrial eDNA ratios. However, the duration and diffusion distance of high concentrations of eDNA produced by spawning activities in water bodies are still unknown, making it challenging to develop an accurate and effective survey plan to monitor spawning activities. No long-term field survey currently uses eDNA methods to monitor fish spawning activity. There is no eDNA-based method to estimate whether fish have spawned. This study aimed to establish a method for estimating fish spawning activity based on eDNA surveys. Therefore, a series of studies were conducted as discussed ahead. First, the changes in the nuclear and mitochondrial eDNA concentrations, ratio of nuclear eDNA/mitochondrial eDNA over time, and distance due to fish spawning activities were estimated, and possible eDNA survey plans to monitor fish spawning activities were established. Second, a method for estimating the spawning activities of a single or a few fish species using eDNA data was established, and the method's effectiveness was verified by comparing with that of the traditional method. Third, the spawning activities of multiple fish were monitored simultaneously using quantitative eDNA metabarcoding technology. Finally, the above experimental results successfully established a method for monitoring fish spawning activities using eDNA.

In Chapter 2, *Cyprinus carpio* was used as a target species, and artificial spawning experiments were conducted to investigate the spatiotemporal changes in nuclear and mitochondrial eDNA concentrations during spawning. The results showed that carp spawning activity over the past 24 h could be monitored by eDNA analysis. Additionally, when carp spawning activity occurs once, spawning activity can be successfully monitored by measuring the nuclear DNA concentration or the nuclear eDNA/ mitochondrial eDNA ratio with a probability of approximately 50–75% based on a sampling plan of sample collection every 100 m and 24 h. Thus, the spawning activity of aquatic species can be estimated with high spatial and temporal accuracy using eDNA analysis.

In Chapter 3, to verify the feasibility of estimating fish spawning activities through eDNA technology, eDNA surveys and traditional surveys were performed for 2 y in a reservoir. Traditional surveys estimated *Micropterus salmoides* and *Lepomis macrochirus* spawning activities from fish body length and daily otolith rings. The eDNA survey used *C. carpio*, *M. salmoides*, and *L. macrochirus* as

target species. A method was established to estimate the spawning activity of the three fish species using the outlier of the eDNA concentration and nuclear eDNA/mitochondrial eDNA ratio. The traditional and eDNA survey results were consistent to a certain extent, which validated the feasibility of estimating fish spawning activities through eDNA surveys.

In Chapter 4, to estimate the spawning activity of whole fish assemblages in a specific water body, a quantitative metabarcoding approach was applied to quantify the eDNA concentrations of all fish species. The results showed that the detected fish species of the quantitative eDNA metabarcoding approach were highly consistent with the historical results of the traditional survey. The spawning activities of 13 fish species were estimated using the method established in Chapter 3. Most spawning activities were consistent with the traditional knowledge of fish spawning periods. These results showed that quantitative eDNA metabarcoding helps monitor the spawning activities of multiple fish species.

Through a series of experiments, an effective survey framework for monitoring fish spawning activity through eDNA was established. This is a complete survey framework for monitoring fish spawning activity based on eDNA, including sample collection, data collection, and data analysis. A sample collection plan was established through the phenomenon of eDNA gradually reducing over time and distance following spawning activity. Taking advantage of the rapid increase in the concentration and ratio of eDNA in fish spawning activities, a method for estimating the spawning activities of a single- or a few fish species was established. Through quantitative metabarcoding technology, the difficulty of simultaneously estimating the spawning activities of multiple fishes is solved. Comparisons with traditional methods and historical records also showed the effectiveness of the eDNA survey framework.

This survey framework is based on eDNA and does not rely on traditional surveys. Although the eDNA-based survey framework for estimating fish spawning activities established in this study still has some drawbacks, such as high concentrations of eDNA resulting from fish gathering can potentially be misinterpreted as spawning activity, and long-term eDNA sampling required to detect the outlier value of the eDNA concentrations. However, in contrast to the traditional deleterious, injurious methods, this framework does not require the collection of fish individuals. Therefore, it does not cause harm to fish

and does not contribute to fish mortality from the survey process. This framework only requires small water samples for a single sampling and does not rely on traditional fishing activities such as drift nets and electrofishing. Therefore, it does not interfere with the biological activities of fish and is an eco-friendly survey method. The method established in this study can effectively reduce field investigation workload and sensitively grasp the spawning time and location of invasive fish and is suitable for monitoring the spawning activities of rare fish. This will deepen the understanding of fish ecology and provide critical information for fish conservation and management.

［課程博士用］

論文審査の結果の要旨

氏　　名	呉　盧漢		
論文題目	Estimating the spawning activities of fish species using environmental DNA （環境DNAによる魚類繁殖活動の推定）		
判　　定	合　　格・不　合　格		
審査委員	区　分	職　名	氏　　名
	主　査	教授	源 利文
	副　査	教授	丑丸 敦史
	副　査	教授	近江戸 伸子
	副　査	教授	高見 泰興
	副　査	京都大学 教授	土居 秀幸

要　　旨

本博士論文では，水中の環境DNA情報を利用して，魚類の繁殖行動をモニタリングする手法の開発について述べられており，実験池を用いて決定された調査戦略を用い，野外の水域において特定の種の繁殖行動をモニタリングする手法，および生息する全ての魚種の繁殖行動を網羅的に把握する手法の開発に成功したことを報告している．さらに，それらの結果に基づき，環境DNA分析によって魚類の繁殖行動をモニタリングするためのフレームワークを提案した．

本論文は５章構成であり，全体を通した序論である第１章，野外の実験池において繁殖活動による環境DNAの濃度などへの時空間的な影響を明らかにした第２章，ダム湖における長期的なモニタリングに基づいて３種の魚種の繁殖行動を明らかにできることを実証的に示した第３章，同じダム湖において多種の繁殖行動をまとめて推定することが可能であることを示した第４章，以上の個別研究で得た結果の総括を行うとともに環境DNA分析に基づく魚類の繁殖行動を把握するための研究フレームワークを示した第５章，謝辞，引用文献，補遺からなる．

第１章では，環境DNAを用いて生物の繁殖行動をモニタリングする手法に関する現在までの進展を総括し，これまでに野外の自然水域に適用された研究例がほとんどないことや，環境DNA分析によって得られるデータの時空間的解像度に関

する研究が不足していることを指摘し，本論文の位置づけを説明している．第２章では，野外の実験池を用いた実験により，コイの繁殖行動時に放出される高濃度の環境DNAの時空間的な挙動を明らかにし，その結果から，野外調査を行う際のサンプリング地点間の距離やサンプリング頻度などの一般的な戦略を決定した．第３章では，福島県の三春ダムの前貯水池を調査地として，２年間にわたって得た環境DNAサンプルを解析することで，コイ，ブルーギル，オオクチバスの３魚種の繁殖のタイミングや場所を推定できることを示すとともに，通常の採捕調査の結果から得られた実際の繁殖のタイミングと，環境DNA分析によって推定されたタイミングが概ね一致することを示した．第４章では，第３章で得た環境DNAサンプルを用い，定量的環境DNAメタバーコーディング手法を用いることで，生息する全ての魚種の繁殖行動を網羅的に明らかにすることが可能であることを示した．第５章では，これらの結果を総合し，環境DNA分析によって魚類などの水中生物の繁殖行動をモニタリングするために包括的なフレームワークを提案した．

本論文では，これまでごく限定的に示されてきた環境DNA分析による水中生物の繁殖行動モニタリングが野外においても可能であることが実証的に示されており，新規性の高いものである．また，分析結果を元にした繁殖期推定モデルについても，新たな統計モデルを自ら開発しており，その研究手法には独創性が認められる．本論文で示した環境DNA分析による繁殖行動モニタリングの応用可能性は広く，希少種の保護や外来種のコントロールなどを通じた生態系の保全に役立つことが期待される．本論文で開発された手法は新たな生態系モニタリング手法として有用であり，人間環境学の観点から高く評価できる．

本論文を構成する各章(第２～４章)は個別の投稿論文としてまとめており，第２章はEcological Indicators誌（査読あり），第３章はFreshwater Biology（査読あり）にて出版済みである．また，第４章は投稿中である．下記に既に公表されている論文の詳細を示す．

Wu, L., Yamamoto, Y., Yamaguchi, S., Minamoto, T. (2022) Spatiotemporal changes in environmental DNA concentrations caused by fish spawning activity. Ecological Indicators 142, 109213.

Wu, L., Wu, Q., Inagawa, T., Okitsu, J., Sakamoto, S., Minamoto, T. (2023) Estimating the spawning activity of fish species using nuclear and mitochondrial environmental DNA concentrations and their ratios. Freshwater Biology 68, 103-114.

よって，学位申請者の呉盧漢氏は，博士（理学）の学位を得る資格があると認める．

レフェリー付きの論文の発表について，記載すること．