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Title	Understanding mechanisms of formation and maintenance of fish biodiversity using environmental DNA [an abstract of entire text]
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Description	この博士論文全文の閲覧方法については、以下のサイトをご参照ください。 https://www.lib.hokudai.ac.jp/dissertations/copy-guides/
Degree Grantor	北海道大学
Degree Name	博士(農学)
Dissertation Number	甲第15774号
Issue Date	2024-03-25
Doc URL	https://hdl.handle.net/2115/92632
Type	doctoral thesis
File Information	YATSUYANAGI_Tetsu_summary.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

博 士 論 文 の 要 約

博士の専攻分野の名称： 博　士（農学）　　氏名 Tetsu Yatsuyanagi

学　位　論　文　題　名

Understanding mechanisms of formation and maintenance of fish biodiversity using environmental DNA

環境 DNA に基づく魚類の生物多様性形成・維持機構に関する研究

How diverse are organisms underwater, and how can we understand them? The diversity of macro-organisms in aquatic ecosystems is predominantly composed of fishes with various ecology occupying various habitats, ranging widely from freshwater to the sea. However, fish diversity remains largely overlooked, with the status of 30% of all described species still unassessed (IUCN 2023). Consequently, there is an imminent necessity to implement effective biodiversity assessment approaches for various fish species including both freshwater and marine fishes that have proven challenging to capture through conventional methods. A promising approach is the environmental DNA (eDNA) method, which utilizes DNA in environmental media such as water. Over the past decade, the eDNA method has rapidly evolved as a non-invasive technique for monitoring species distributions, enabling comprehensive surveys of fish fauna across wide-ranging and multiple sites, both in freshwater and marine environments. However, there have been substantial challenges to address genetic diversity, species diversity, and diversity of life history traits such as migration patterns in fishes. In this thesis, I aimed to understand formation and maintenance of fish diversity utilizing the eDNA method in freshwater and marine environments.

Specifically, I tackled three challenges: intra-specific phylogeography, the coexistence of closely related species, and a comprehensive understanding of migratory life histories.

In Chapter 1, I conducted eDNA-based phylogeographic study on Siberian stone loach (*Barbatula toni*), a primary freshwater fish commonly found in northern continental Far East, Sakhalin, and Hokkaido. The objective was to unveil the history of genetic diversity formation in relation to geography, leveraging the characteristics of the primary freshwater fish with restricted dispersal across different rivers. The eDNA application for detecting intra-specific genetic diversity has many risks of false positives such as sequencing errors. In this study, utilizing a novel PCR primer set and bioinformatic processing to mitigate such errors, a total of 50 haplotypes were detected throughout Hokkaido, revealing two regionally restricted, genetically distinct lineages. By integrating the eDNA method with conventional molecular techniques, I found that this phylogenetic divergence possibly arose from the long-time isolation between Hokkaido and Sakhalin populations over a million years. Subsequently, it is estimated that Sakhalin lineages colonized in northern Hokkaido when Sakhalin and Hokkaido had been connected by a land bridge in the recent past, resulting in the north-south phylogeographic structure in Hokkaido observed in this study. These results illustrate how the eDNA method can enhance our insights into the historical process of the formation of genetic diversity.

In Chapter 2, I targeted on the highly diverse and closely related rockfishes (genus *Sebastes*), aiming to reveal geographic patterns of species diversity and the factors contributing to the maintenance of their diversity in the marine environment. The diversity of the *Sebastes* species has been overlooked in previous eDNA studies due to the high genetic similarity between species, making identification difficult using standard metabarcoding assay. Utilizing a newly-modified, genus-specific eDNA metabarcoding

assay, 14 *Sebastes* species were detected in 43 coastal sites in northern Japan. Contrary to prior expectations based on passive dispersal in the sea, distinct distribution boundaries for each species were observed. Moreover, areas with larger depth gradients exhibited higher species richness for *Sebastes* species, suggesting the importance of vertical habitat partitioning for multispecies coexistence. These findings highlight the contribution of strong environmental preferences to the maintenance of species diversity in marine environments.

In Chapter 3, I conducted a review of studies targeting migratory freshwater fish using eDNA. Migratory fishes have their own life histories that efficiently link multiple ecosystems. Therefore, comprehensive understanding of migration ecologies is essential not only for species conservation but also for biodiversity maintenance and ecosystem management. The objective was to reveal the extent to which the ecology of highly elusive fish has been addressed and to identify overlooked aspects in the current eDNA-based studies. A literature review of 51 papers revealed that the habitat uses of 69 species with a variety of migratory types have been explored using eDNA. Significantly, more than 60% of them were listed on the IUCN Red List. While numerous achievements have been demonstrated especially on endangered species, a critical issue has become apparent: less than 10% of the research focused on marine migrations. Given this fact, I presented noteworthy case studies that have addressed the ecology of migratory fishes in both marine and freshwater systems .

In Chapter 4, I conducted an eDNA survey for understanding marine migration of an endemic anadromous fish, Shishamo smelt (*Spirinchus lanceolatus*). As revealed in Chapter 3, the eDNA method proves effective for monitoring migratory fish; however, there is a shortage of case studies applying it in marine environments. In this study, using species-specific quantitative PCR, I examined 1) seasonal habitat changes in coastal marine regions

and 2) environmental factors potentially driving the marine migration of this species. The eDNA concentrations along a 100 km-long coastline exhibited spatiotemporal variation, suggesting that this species shifts their habitat away from nearshore areas between spring and summer. We also found a significantly negative association between the eDNA concentration and sea surface temperature. That finding suggests that the offshore migration of this species is associated with increased sea surface temperature. This study reveals new aspects of *S. lanceolatus* life history in coastal regions. Together with the previous eDNA study on the freshwater migration of *S. lanceolatus*, this study illustrates the potential of eDNA techniques for understanding the whole life history of migratory organisms.

In summary, this thesis provides innovative insights into the formation and maintenance of species, genetic, and life-history diversity in fishes. Through technical evaluation, the eDNA methods emerges as a tool capable of unveiling perspectives beyond conventional approaches, irrespective of the characteristics of aquatic environments (i.e., freshwater or seawater) or species lifestyles. These findings will contribute to unraveling the dynamic biodiversity in aquatic ecosystems, which largely remains a frontier.