



HOKKAIDO UNIVERSITY

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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 fish remains largely overlooked, with the status of 30% of all described species still unassessed. Consequently, there is an imminent necessity to implement effective biodiversity assessment approaches for various fish species 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. In this thesis, I focused on three different types of fish species/groups to address new perspectives on their diversity formation and maintenance, utilizing the eDNA technique. Specifically, I tackled three challenges: intra-specific phylogeography, the coexistence of closely related species, and a comprehensive understanding of migratory life histories.

In the freshwater system, I conducted eDNA-based phylogeographic study on *Barbatula toni*, a common and unique species in 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. Using a novel eDNA assay, a total of 50 haplotypes were detected throughout Hokkaido, revealing two regionally restricted, genetically distinct lineages. These results illustrate how eDNA assay can enhance our understanding of the formation of genetic diversity.

In the marine system, I focused on the highly diverse and closely related rockfishes (genus *Sebastes*), aiming to reveal geographic patterns of species diversity and the factors contributing to their maintenance. Utilizing a genus-specific eDNA metabarcoding assay, 15 *Sebastes* species were detected 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, 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.

Lastly, I conducted a review of studies targeting migratory fish using eDNA. The objective was to reveal the extent to which the ecology of highly elusive fish has been addressed and to identify overlooked aspects. A literature review of 51 papers revealed that the habitat uses of 69 species with a variety of migratory types were 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 the fact, I presented noteworthy case studies that have addressed the ecology of migratory fishes in both marine and freshwater systems.

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 technique emerges as a tool capable of unveiling perspectives beyond conventional approaches, irrespective of the characteristics of aquatic environments or species lifestyles. These findings will contribute to unraveling the dynamic biodiversity in aquatic ecosystems, which currently remains a frontier.