Summary

In the natural environment, there exist complex relationships between organisms and the environment as well as among organisms. To conserve biodiversity and ecosystems with the development of human society, we must understand organism distributions and biomass/abundance to simulate and predict their dynamics.

Environmental DNA (eDNA) methods have been developed to detect the distribution, abundance, and biomass of organisms in various environments. eDNA methods can be used to detect target species as well as to estimate their biomass and abundance. However, various factors such as sampling season, temperature, pH, salinity, and UV can influence biomass and abundance estimations from eDNA. eDNA generally degrades quickly in the environment; thus, understanding eDNA degradation is critical for eDNA evaluation. Several aspects of the general behavior of eDNA in water remain unclear, especially the state and degradation of eDNA. Many experiments have been conducted on the different states and degradation rates of eDNA under various conditions. In most cases, the eDNA degradation curves declined exponentially, and the degradation rates were fast, but occurred in less than a week. However, other studies have found that eDNA concentrations can decay below the detection limit in less than a week. Additionally, water conditions such as salinity, temperature, and pH can influence the eDNA degradation rate.

Simulation techniques have been used in ecology, particularly for estimating ecological population and community dynamics. The abovementioned issues related to eDNA surveys can improved through modelling and simulation. For example, simulating the spread of eDNA in water may provide a more detailed estimate of the habitat of

released species. Additionally, by predicting the amount of eDNA degradation, we can estimate, for example, how much eDNA will be degraded by the time the water sample reaches the laboratory.

Understanding eDNA states and degradation is essential for the effective sampling and storage of eDNA, and may provide pertinent information to better interpret the results of species distribution, abundance, and biomass estimations. In some cases, eDNA has not been detected, despite confirmation of the habitat of organisms. It has been indicated that false negatives may involve eDNA degradation in the environment and eDNA sample processing, such as water sample transport.

In this study, the characteristics and dynamics of eDNA released by organisms into the environment were verified through experiments and simulations. The aim of this doctoral thesis was to refine the relationship between the characteristics and degradation of eDNA from macroorganisms and to obtain clues to mitigate and eliminate uncertainties related to eDNA detection and quantification. In this thesis, I studied the degradation of eDNA from multiple perspectives, using a meta-analytical approach, which is described in Chapters 2. I also used an experimental approach, which is described in Chapters 3 and 4.

I investigated eDNA degradation using a meta-analytic approach based on previous data and performed eDNA degradation prediction simulations. Our meta-analysis results showed that eDNA degradation was accelerated at higher water temperatures and longer amplicon lengths. Previous studies have assumed that water temperature does not directly affect eDNA degradation, rather indirectly affecting it through enzymatic hydrolysis by microbes and extracellular nucleases. At high temperatures, with increasing activity of microorganisms and extracellular enzymes,

eDNA in water degraded more quickly.

I found that the eDNA derived from cells and fragmented DNA declined exponentially after being added to both seawater and pond water samples. The eDNA from resident species showed similar behavior to the eDNA derived from cells. In most cases, a simple exponential model can be used to evaluate the degradation. Increased salinity in the saline sample had no effect on the DNA detection. The degradation rates of the saline pond and pond samples were significantly different. Furthermore, the degradation rates of the diluted pond and pond samples were significantly different. For all sources, the reduction in degradation rate preserved more DNA than the disadvantage caused by reducing the initial concentration to 1/10.

In this study, I examined, validated, and discussed eDNA analysis from multiple perspectives using simulation and modelling through meta-analysis and degradation experiments. Although studies relating to eDNA characteristics and dynamics increase yearly, their number is small compared to the number of overall eDNA studies. However, its practical applicability is still in its infancy, both because of the uncertainty of detection sensitivity and quantification accuracy of eDNA in the field, as well as the lack of information on populations other than presence/absence and/or abundance. Elucidating these features will improve the usefulness, practicality, and reliability of eDNA analysis as a tool for biodiversity and ecosystem monitoring and stock assessment in the future. More, and a deeper understanding of, basic information about eDNA will improve eDNA analysis methods and enable researchers to maximize the potential of future eDNA methods. The outcomes of this study, especially our simulation and models, will add to the basic information on eDNA and contribute to the elucidation of the characteristics and degradation mechanism of eDNA. The findings presented in this thesis provide important

groundwork for innovating eDNA analysis for biodiversity monitoring, ecological assessment, and resource management in the future.