令和３年度　環境科学院　修士論文内容の要旨

Development of eDNA detection system for evaluating abundance and reproduction in winter for freshwater mussels (*Buldowskia iwakawai*) in the Ishikari river floodplain

(石狩川氾濫原における淡水二枚貝 (*Buldowskia iwakawai*) の生息数および冬季再生産把握に向けた環境DNA手法の確立)

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Freshwater mussels (Order Unionoida) can provide crucial ecological functions such as habitat provisioning and mediating material/nutrient cycles while many species are endangered due to habitat destruction and water contamination. Freshwater mussel larvae are characterized by an obligate parasitic stage during which larvae emitted from females parasitize on host fish for a period before initiating juvenile life stage. The monitoring of mussel life cycles including reproduction activities is among top priorities for approaching freshwater mussel conservation activities. Targeted species *Buldowskia iwakawai* is a Japan native freshwater mussel species distributed in Ishikari Floodplain oxbow lakes. The reproductive failure of *B. iwakawai* has been recently reported based on observations that juvenile individuals can hardly be found. This study aims to develop an environmental DNA (eDNA) method to monitor *B. iwakawai* more efficiently including winter breeding season under frozen surface water. Two questions were addressed: 1) Can eDNA reflect hand-collection abundance? 2) Can eDNA detect gravidity (status of larvae carrying and developing) of mussels in controlled-lab environments and fields?

Fourteen lakes were chosen as study sites. First, based on COI gene from previously collected tissues, a species-specific eDNA detection system was designed using TaqMan and SYBR Green methods, and applied to detect eDNA concentrations in real-time quantitative PCR. This detection system was applied to environmental water samples collected in October 2020 and compared with previously reported hand-collection abundance. Second, gravid and non-gravid individuals were regularly taken back from an intensively monitored site, reared in tanks with tap water (mesocosm experiments), and the detection system was applied to tank water samples. Furthermore, a monthly environmental water sampling was performed from November in 2020 to June 2021 covering reproduction season with surface water being frozen. Supplementarily, the efficiency of detection system in different water types (tap and natural water), size-dependent metabolic rates, and field gravidity stage progression were examined.

The eDNA concentration in 14 sites positively correlated with mussel abundance, which proves the ability of eDNA as a tool for quantifying mussel density. The eDNA concentration was significantly higher for gravid mussels in February mesocosm experiment, which was presumably related to an increased metabolic rate, indicating the applicability of eDNA method to detect specific gravidity stages. The predicted eDNA increase synchronized with the gravidity progression was not observed whereas an exceedingly high concentration was observed in October and November in the field. This discrepancy might be related with the environmental factors specific to the field such as lake water quality variations, water stratification, and lake hydrology (dilution and seasonal turnover). Furthermore, an unexpectedly high concentration suggests that eDNA method has the possibility to detect sperm releasing activity of male individuals in autumn season when lakes are better circulated. A water sampling method that can increase eDNA capture rate and a suitable eDNA extraction and amplification protocol that can avoid PCR inhibitors should be considered to improve the eDNA detection possibility in icy waterbodies.