令和 4 年度 環境科学院 修士論文内容の要旨

Development of species-specific eDNA technology to detect and quantify hyporheic invertebrates in rivers

（河川飽和間隙水域における無脊椎動物の検出のための種特異的環境 DNA 技術開発）

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In river ecosystems, hyporheic zone (HZ) provides critical habitat for many invertebrates and is important for maintaining biodiversity. Many factors influence invertebrates' activities in HZ, including river gradient, sediment load, and water temperature. However, compared to surface water, HZ is more difficult to be directly observed, and there are relatively few previous studies and limited scientific understanding. Theoretically speaking, environmental DNA (eDNA) technology can be used to analyze environmental water samples to understand the habitat conditions and distribution of organisms and is expected to be an effective method to significantly advance our understanding of HZ biota.

The objective of this study was to develop a technique for detecting the abundance of *Alloperla ishikariana* (Order: Plecoptera), a model species of HZ aquatic insects, using species-specific eDNA technology under controlled and field environment. This species accounts for >70% of the insect biomass in the HZ of some gravel-bed rivers and is therefore an excellent indicator of the ecological environment. This study was conducted in 17 rivers in Hokkaido, Japan. Surface water samples were collected in these rivers during different seasons (March 2022 and November 2022) when they were at aquatic larval stages, as well as from controlled indoor tanks with different abundance of the individuals reared for 4-5 days. In addition, species specific primer-probe sets designed for this species were created and DNA copy numbers were quantitatively estimated.

Field-water eDNA of this species was detected only in November, that is, after their oviposition and egg hatching, but its concentration didn't correlate with their estimated individual abundance. In the indoor tank experiments, their presence/absence and abundance-dependent increases of eDNA was detected. Additional tank experiment of the influence of sediment composition and quantity on the eDNA detection of this species showed that fine sediments substantially decrease eDNA concentration. Field water detection in November was possibly related to the species life-history with relatively high abundance of newly hatched individuals in the benthic zone. Although the mechanism remains unknown, the presence of fine sediment and their behaviors of "being buried in sediment" might have substantially lower the probability of detection of this species in the field environment. Overall, the species-specific eDNA technology has the potential to provide a more convenient and economical idea for future exploration of HZ, but more sophisticated and advanced processing is necessary.