

Ecological study of salmonid fish and its cold-water disease pathogen *Flavobacterium psychrophilum* using the environmental DNA method in natural rivers

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1. Introduction

The death of salmonid fish by cold-water disease pathogen *Flavobacterium psychrophilum* mainly occurs in hatcheries, and little is known about the disease in natural rivers. In Ayu, on the other hand, horizontal infection from hatchery fish to native fish has been reported (Ueda et al., 2000). In addition, it has been reported that the parent chum salmon captured in a river in Hokkaido has cold-water disease pathogen colonization within their body cavity fluid (Hatayama, 2013). They suggest that there is a possibility that horizontal infection or small-scale outbreak of the cold-water disease might have occurred in salmonid fish in natural rivers. In this study, we develop the technology aiming at quantifying *F. psychrophilum* in the natural river using the environmental DNA method and examining the relationship between salmonid fish migration and *F. psychrophilum* during spawning season.

2. Methods

We designed *F. psychrophilum* specific primers and optimized the environmental DNA method (eDNA-method, hereafter) for detecting the bacteria with the target culture medium using two kinds of filter paper. Outdoor sampling was conducted in Chitose and Naibetsu river from May 2015 to August 2016. During salmon spawning season (September and October) in 2016, we collected water samples at high frequency and observed the number and the sex of running fish. After collecting these samples, quantitative PCR was carried out by the SYBR method for *F. psychrophilum*, and the probe method for chum salmon (*Oncorhynchus keta*).

3. Results

F. psychrophilum from the water sample of the culture solution was detected using the eDNA-method, and there was a correlation between the number of viable bacteria in the water sample and the amount of DNA detected. In addition, a positive correlation was also found between the DNA amount of chum salmon and the DNA amount of *F. psychrophilum* in the Chitose river samples. In Naibetsu river, on the other hand, the correlation was weak. DNA from the two species were detected even when living salmon and dead body were few in the study period.

4. Conclusions

In Naibetsu river, although the absolute number of salmon was smaller than in Chitose river, the DNA amount of *F. psychrophilum* was large. This result might be caused by releasing body cavity fluid included *F. psychrophilum* at egg laying, regardless of the number of living or dead salmon.