

Stability of environmental DNA methylation and its utility in tracing reproductive activities of fish

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Abstract

1. The use of environmental DNA (eDNA) as a new method of ecological monitoring is widely applied. Although eDNA can provide important information on the distribution and biomass of particular taxa, an organism's DNA sequences remain unaltered throughout its existence, which complicates identifying crucial events, including reproduction, with high accuracy. We thus examined DNA methylation as a novel source of information from eDNA, considering that methylation patterns of eggs and sperm released during reproduction differ from those of somatic tissues. 2. Despite its potential applications, little is known about eDNA methylation, including its stability and methods for detection and quantification. Therefore, we conducted tank experiments and performed methylation analysis targeting 18S rDNA through bisulfite amplicon sequencing. 3. Methylation of eDNA was not affected by degradation and was equivalent to the rate of genomic DNA from somatic tissues. Unmethylated DNA, which is abundant in the ovary, was detected in eDNA during reproductive activity of fish. 4. These results indicate that eDNA methylation is a stable signal reflecting genomic methylation and demonstrate that germ cell-specific methylation patterns can be used as markers for detecting reproductive activity.

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