Investigating the effect of storm on estimating aquatic insect community in water stream using environmental DNA

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1. INTRODUCTION

Estimating and measuring aquatic insects' community in water stream has become one of the fundamental points in conserving ecosystem in water stream. In recent years, the usage of environmental DNA metabarcoding from water samples has rapidly grow due to the fact that it is much more easier and energy conservative compared to the traditional survey technique. Environmental DNA refers to DNA that is released from the body of an organism and exists in water, and is thought to be derived from the skin fragments and excrement of the organism. By sampling the water and analyzing the DNA contained in it we can identify the number of species habituating those area. Some studies also reported that environmental DNA also can detect aquatic species at relatively low densities. Speaking of that, it is said that conducting survey to determine the presence of an insects in a river system might be the easiest way to measure the water quality of a river system.

Despite of the fact that environmental DNA metabarcoding can improve the accuracy of species detection for aquatic environments, natural phenomenon such as storm or rainfall might have the alter the final result of estimating aquatic insect's community using environmental DNA. There is less study that investigate the effect of this natural phenomenon to the environmental DNA metabarcoding method. The total understanding between environmental DNA analysis and natural phenomenon such as storm is needed to utilize environmental DNA as a transformative tool for monitoring and estimating aquatic biodiversity in the future. Because of that, in this study we will investigate the effect of rainstorm can bring in using environmental DNA metabarcoding method to estimate aquatic insects' community in water stream.

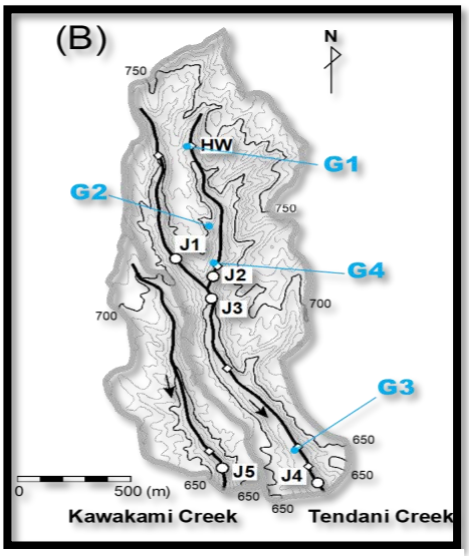

Figure 1 Sampling site

2. METHODS

2-1 Water Sampling and filtration

Water sample was collected 4 sites (G1, G2, G3, G4) along the Tendani creek, Hiruzen Experimental Forest of Tottori University, Okayama, Japan (Figure 1). The 3 of 4 sites (e.g., G1, G3, G4) were located along main stream. The water in G2 site contained not only river water but also spring one. The first water sample was collected in 16th September 2017 between 8:40-9:20 in the morning. Second water sample were collected in 18th September 2017. Typhoon Talim passed the study site on 17th September 2017 (Figure 2). Collected water samples (300ml) were filtrated using membrane filter (ADVANTEC, pore size 0.2μm).

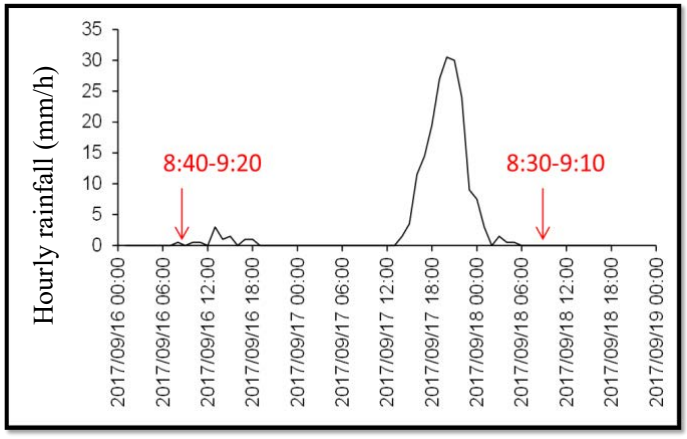

Figure 2 Hourly rainfall and water sampling time

2-2 DNA Extraction & PCR Method

We extracted DNA from the filters by Phenol-chloroform extraction and Isopropanol precipitation method. Each DNA was amplified Cytochrome Oxidase I (COI) region on mitochondrial DNA by Polymerase Chain Reaction. The reaction solution was 15.5μL of PCR Grade Water, 4μL 25 mM dNTP (TaKaRa), 10μL of 5x Phusion GC Buffer (New England), 10μM forward primer (BF1) 5μL and 10μM reverse primer (BR2) 5μL (citation), 10%

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Tween20 5μL, a total of 0.5μL Phusion High-Fidelity DNA Polymerase (New England) and 5μL of 10 times diluted DNA were mixed. PCR steps were conducted by PCR Thermal Cycler Dice (TaKaRa). PCR cycling conditions were 95°C 3 min, followed by 40 cycles 95°C for 30 seconds, followed by 50°C for 30 seconds and 72°C for 45 seconds, then followed by extension at 72 °C for 5 minutes. We ran the PCR products through electrophoresis method to confirm the final product of the PCR product using Mini Agarose Gel Electrophoresis apparatus.

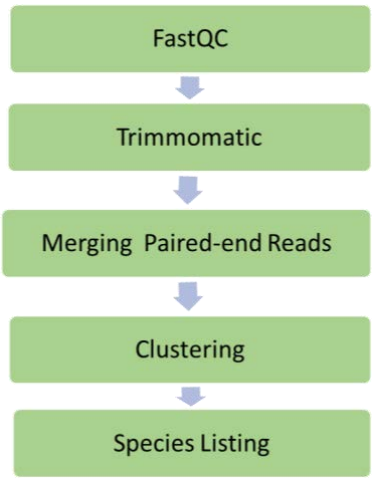

Figure 3 pipeline for NGS data analysis

2-3 Next-generation sequencing (NGS) data analysis

The amplified PCR product was sent to bio-engineering lab to undergo next-generation sequencing (NGS) using MiSeq system (Illumina) and MiSeq reagents v3 (Illumina). The sequence data from NGS was analyzed to list the number of species of each sampling point and each date following the pipeline represented in Figure 3. We employed FastQC to confirm sequence quality and Trimomatic v0.39 (Bolger et al 2014) to remove primer region, low-quality region and low-quality sequences. Then, PEAR v0.98 (Zhang et al 2014) was used to join paired-end reads. Operational Taxonomic Units (OTUs) were clustered with 97% homology using Claident. We later group similar unique read into OUT in clustering step and using the Basic Local Alignment Search Tool (BLAST), the species was subsequently identified by DNA barcoding.

3. RESULT AND DISCUSSION

We performed the DNA meta-barcoding of environmental DNA collected before/after a typhoon event in Tendani creek, Okayama, Japan. Larger number of OTUs were detected after the typhon in all sites (Figure 4). This phenomenon can be conducted by two reasons; 1) the transportation of organic matter from land and 2) the turbulence of sediments in river bottom. In general, rain water can carry a lot of organic materials containing animal DNA from land into river water. The typhoon in the study time could also transport the organic materials and we could detect not only aquatic DNA but also inland one by DNA meta-barcoding. Another possibility reason is related to water turbulence in stream bottom. Typhoon event can disturb bottom sediments and organic materials in sediments can be appeared into river water. Therefore, DNA from animals live in sediment also could be detected. In conclusion, it could be more efficient to collect water after storm event to order to investigate species diversity using environmental DNA.

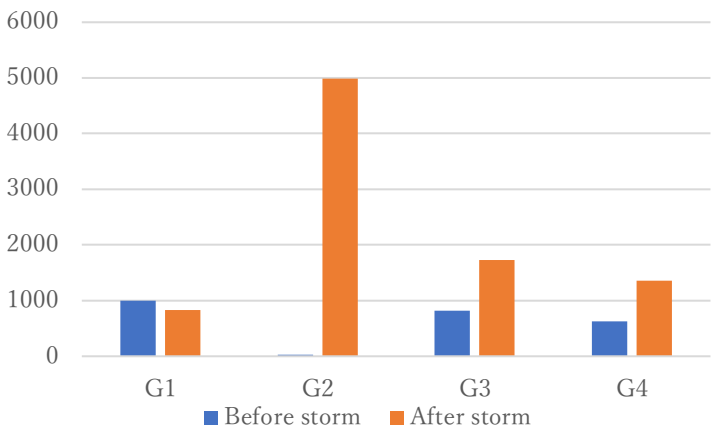

Figure 4 Number of OTU in each sampling point

REFERENCES

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina Sequence Data. *Bioinformatics*, btu170.

Hadley, Debbie. "What Aquatic Insects Tell Us About Water Quality." ThoughtCo, Aug. 12, 2019, thoughtco.com/water-monitoring-and-aquatic-macroinvertebrates-1968647.

Olds, B. P. *et al*. Estimating species richness using environmentalDNA. *Ecol. Evol.* **6**, 4214–4226 (2016)

Pilliod, D. S., Goldberg, C. S., Arkle, R. S. & Waits, L. P. Factors influencing detection of eDNA from a stream-dwelling amphibian. *Mol. Ecol. Resour.* **14**, 109–116 (2014)

Zhang J, Kobert K, Flouri T, Stamatakis A. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics. 2014;30(5):614–620. doi:10.1093/bioinformatics/btt593

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