

### 中央研究院生物多樣性研究中心 Biodiversity Research Center, Academia Sinica

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### **Zooplankton Metatranscriptomics**



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#### Abstract

DNA metabarcoding is a widely used molecular method for biomonitoring of zooplankton communities. This PCR-based method amplifies a target gene region from genomic DNA (gDNA) before nextgeneration sequencing (NGS). However, DNA metabarcoding has methodological uncertainties that limit its capacity to reflect the true diversity of complex zooplankton communities. Methodological biases related to the co-detection of environmental DNA (eDNA) when using gDNA templates may also affect the accuracy of downstream analyses by adding more background noise to the sequence data. The co-amplification of mitochondrial-like templates in the nuclear genome, also called nuclearencoded mitochondrial (NUMT) pseudogenes, when using genomic DNA templates may lead to inflated diversity estimates. NUMT pseudogenes are well documented in various metazoan taxa, especially in copepods, which have large nuclear genomes. Most importantly, PCR-amplification bias may happen when primers fail to bind effectively to the target gene sequences of specific taxa, leading to inaccurate estimates of community diversity and composition. Given these methodological uncertainties, a molecular approach bypassing PCR and using RNA instead of gDNA as a template is an ideal alternative method for monitoring zooplankton communities. RNAbased methods that capture messenger RNA (mRNA), such as metatranscriptomics, may be less prone to biases when characterizing zooplankton communities. The isolation of mRNA transcripts rather than gDNA reduces the chance of NUMT pseudogene contamination because are not transcribed into mature pseudogenes mRNA. Moreover, metatranscriptomics does not require amplification of a target gene region using PCR, avoiding biases related to primer binding efficiency. Metatranscriptomics has been useful in advancing many aspects of plankton research but remains underused in zooplankton studies, which has hampered progress in this field compared with phytoplankton and microbial research. The doubts regarding the use of RNA-based methods may be rooted in misconceptions regarding the difficulty of sample preservation, storage, and bioinformatics procedures. Consequently, the performance of metatranscriptomics at characterizing zooplankton community samples has not been rigorously validated, especially in comparison to DNA metabarcoding and morphological analysis. We assess the ability of metatranscriptomics to estimate the diversity and composition of zooplankton using both mock communities and field-collected samples. The results of this study may help advance the use of metatranscriptomics in zooplankton monitoring.