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A comparative analysis of real-time quantitative PCR and metabarcoding methods for eDNA-based detection of the toxic dinophyte *Alexandrium tamiyavanichii* (Dinophyceae)

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ABSTRACT

The marine dinophyte *Alexandrium tamiyavanichii* is a toxigenic species that produces a group of neurotoxins that is responsible for paralytic shellfish poisoning in humans. Early detection of the species is essential for efficient monitoring. Harmful microalgal monitoring systems have evolved over the years with the advent of environmental DNA (eDNA)-based species detection techniques. In this study, eDNA samples were collected from a large-scale sampling covering the southern South China Sea. The sensitivity and specificity of metabarcoding of the V4 and V9 18S ribosomal DNA barcodes by high-throughput sequencing (HTS) were compared to the species-specific real-time qPCR targeting the *A. tamiyavanichii* ITS2 region. Environmental samples were screened for *A. tamiyavanichii* by qPCR ($n = 43$) and analyzed with metabarcoding ($n = 30$). Our results revealed a high occupancy profile across samples for both methods; 88% by qPCR, and 80–83% by HTS. When comparing the consistency between the two approaches, only two samples out of 30 were discordant. The V4 and V9 molecular units detected in each sample were positively correlated with the qPCR ITS2 gene copies (V4, $r_s = 0.67$, $p < 0.0001$; V9, $r_s = 0.65$, $p < 0.0001$), indicating that metabarcoding could be used as a useful tool for early detection of the species. Our results also revealed that the estimation of *A. tamiyavanichii* cell abundances based on the HTS read abundances was comparable to that of the qPCR quantification. For long-term monitoring, metabarcoding could serve as a cost-effective screening of detecting not only single HAB species but also simultaneously detecting a multitude of potentially harmful species, which is valuable in informing the subsequent implementation of species-specific monitoring strategies.

1. Introduction

Harmful Algal Blooms (HABs) occur when specific microalgal species undergo prolific proliferation within aquatic ecosystems, resulting in deleterious consequences for both mankind and the environment. The incidences and geographical expansion of known and emerging HAB species have shown a growing prevalence in regional (Furuya et al., 2018; Yñiquez et al., 2021) and global contexts (Hallegraeff et al., 2021). Contamination of the algal-origin toxins in wild or cultured shellfish mollusks has contributed to various types of shellfish poisoning, including paralytic shellfish poisoning (PSP) – a human illness that is caused by consuming shellfish mollusks contaminated with a family of sodium channel-blocking neurotoxins, Saxitoxins (STXs). STXs bind reversibly to the voltage-dependent sodium channel in the mammalian nervous system and resulted in both neurological and gastrointestinal symptoms (Stevens et al., 2011).

The marine dinophytes in the genera of *Alexandrium*, *Pyrodinium*, and *Gymnodinium* are the main algal producers of PSP toxins (Oshima et al., 1993; Lim et al., 2020). The toxin producers of *Pyrodinium* (*P. bahamense*) and *Gymnodinium* (*G. catenatum*) are monospecific, while of the 32 *Alexandrium* species, about one-third are known to produce PSP toxins (Lundholm et al., 2023). Among them, *Alexandrium tamiyavanichii* is one of the most toxic species, exhibiting a cellular paralytic shellfish toxins (PSTs) content of up to 180 fmol PSTs cell^{-1} (Lim and

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