



Molecular diversity of benthic harmful dinoflagellates on a tropical reef: Comparing natural and artificial substrate sampling methods using DNA metabarcoding and morphological analysis

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ARTICLE INFO

Keywords:

Artificial substrate
BHAB
Benthic
Dinoflagellate
Ciguatera
Gambierdiscus
Ostreopsis

ABSTRACT

Harmful algal blooms in the benthic system (BHAB) are a major environmental problem that has increased worldwide in the context of global climate change. While systematic cell-based BHAB monitoring for risk assessment and early warning systems have been recommended, implementation of a standardized sampling method is challenging owing to the benthic nature of these harmful microalgal taxa. This study investigated the molecular diversity of benthic harmful dinoflagellates in tropical reefs of Perhentian Islands, Malaysia, using artificial substrate (AS) and sampling natural substrates (NS), combined with environmental DNA (eDNA) analysis and high-throughput amplicon sequencing targeting the small subunit (SSU) and large subunit (LSU) rDNA markers. Our results revealed that the AS method effectively captured a representative subset of the benthic dinoflagellate community, with significant taxonomic overlap between AS and NS. Both markers enabled high-resolution detection of BHAB taxa, particularly of *Gambierdiscus* and *Ostreopsis*, which are challenging to identify by light microscopy. The LSU rDNA marker provided finer taxonomic resolution, capturing a broader range of dinoflagellate species. The molecular approach consistently aligned with cell quantification data, supporting AS and DNA metabarcoding as robust methods for BHAB monitoring. The findings highlight the potential of these methods for early detection, especially areas susceptible for ciguatera and BHAB-related poisoning, offering a systematic approach for routine cell-based monitoring.

1. Introduction

Benthic harmful algal blooms (BHABs) have gained increasing scientific attention due to their increasing incidence that linked to human illnesses and their expanding distribution beyond known endemic zones (Berdalet et al., 2012; 2017). The tropical and sub-tropical areas are particularly susceptible, as these regions have faced increased food security risks driven by ocean warming (Tester, 1996; Rhodes, 2011; Berdalet et al., 2017; Tester et al., 2020). Marine environmental changes, particularly the sea surface temperatures, profoundly affect the distribution and proliferation of these toxin-producing benthic dinoflagellate species (Berdalet et al., 2017; Tester et al., 2020, 2022). Key genera responsible for BHABs included *Gambierdiscus*, *Ostreopsis*, *Amphidinium*, *Coolia*, and *Prorocentrum* (Berdalet et al., 2012), all of which are known to produce a variety of bioactive substances

(Hoppenrath et al., 2014). Of these, *Gambierdiscus* and *Ostreopsis* are particularly notable, as they produce biotoxins that alter ecosystem processes and pose serious risks to both human and animal health (Litaker et al., 2017; Larsson et al., 2018; Pavaux et al., 2020). *Gambierdiscus* species produce ciguatoxins (CTXs), a group of neurotoxins that transfer and bioaccumulate through the food web, causing ciguatera poisoning (CP) in human (Scheuer et al., 1967; Anderson and Lobel, 1987). Similarly, certain species of *Ostreopsis* are known for their production of palytoxin analogs (PlTX), including ovatoxins (OVTXs), that responsible for respiratory, dermatological, and ocular syndromes during bloom events, specifically Mediterranean regions (Pfannkuchen et al., 2012; Vila et al., 2016; 2017). Other biotoxins, including okadaic acid (OA) (Tachibana et al., 1981), dinophysistoxins (DTXs), cooliatxin (Holmes et al., 1995), and amphidinolides (Kobayashi et al., 1986, 1988), have been characterized from species of *Prorocentrum*, *Coolia*,

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<https://doi.org/10.1016/j.hal.2024.102795>

Received 25 October 2024; Received in revised form 28 December 2024; Accepted 29 December 2024

Available online 30 December 2024

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