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Toxic bloom of *Pseudo-nitzschia cuspidata* (Bacillariophyceae) and domoic acid contamination of bivalve molluscs in Malaysia Borneo

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ABSTRACT

In March 2018, an algal bloom of *Pseudo-nitzschia* was detected, for the first time, in a semi-enclosed lagoon in Miri, Sarawak, Malaysia Borneo. The plankton samples were collected for cell enumeration and species identification by electron microscopy and molecular characterization. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was performed to detect and quantify the neurotoxin domoic acid (DA) in both the plankton and shellfish samples. The abundance of *Pseudo-nitzschia* cells ranged from 5.6×10^5 to 3.5×10^6 cell L^{-1} during the bloom event. Morphological observation of the cells by transmission electron microscopy showed that the plankton samples comprised a single *Pseudo-nitzschia* morphotype resembling *P. cuspidata*. The ITS2 sequence-structure phylogenetic inference further supported the species identity as *Pseudo-nitzschia cuspidata*. Low levels of DA were detected in the plankton samples, with cellular DA, particulate DA, and dissolved DA of 257–504 fg DA $cell^{-1}$, 676 ng L^{-1}, and 15 ng L^{-1}, respectively. The amount of DA, 8 μg g^{-1} tissue, was found present in the shellfish sample (*Magallana* sp.) which is below the regulatory limit of 20 μg DA g^{-1} tissue. The study documented, for the first time, DA contamination in shellfish that associated with bloom of *P. cuspidata* in the Western Pacific region.

1. Introduction

The pennate diatom *Pseudo-nitzschia* is known to produce domoic acid (DA), a neurotoxin responsible for amnesic shellfish poisoning (ASP) or DA poisoning (reviewed in Bates et al., 2018, Bates et al., 2019). At least 27 of the 58 currently recognized *Pseudo-nitzschia* species have been categorized as DA-producing species (Li et al., 2017; Bates et al., 2018; Huang et al., 2019). In the past decade, vast studies have been conducted to investigate *Pseudo-nitzschia* in terms of the taxonomy, molecular systematics, ecology, and toxicology (Lefebvre and Robinson, 2010; Lelong et al., 2012; Trainer et al., 2012; Bates et al., 2018, 2019). Species of *Pseudo-nitzschia* have been identified based on the variability of frustule morphological characteristics, which was obtained through detailed microscopic investigations of the ultrastructure of cell valves (Lelong et al., 2012; Bates et al., 2018). The informative morphological features included valve shape, densities of fibulae and striae, poroid structures, and cingular bands (as reviewed in Bates et al., 2018). Apart from that, molecular characterization using gene markers has been applied to support the delimitation of novel species (Lundholm et al., 2006, 2012; Amato and Montresor, 2008; Quijano-Scheggia et al., 2009; Lim et al., 2013; Orive et al., 2013; Teng et al. 2014, 2016), including cryptic species such as *P. delicatissima*-*P. arenysensis* (Quijano-Scheggia et al., 2009).

The discoveries of toxigenic species of *Pseudo-nitzschia* have been increasingly reported from the Southeast Asian region (Dao et al., 2006; 2009, 2014, 2015; Teng et al., 2014, 2016), although no toxic bloom of *Pseudo-nitzschia* was recorded thus far. In Malaysia, research attention on *Pseudo-nitzschia* has been focused mainly on the species diversity, taxonomy, and molecular systematics (e.g., Lim et al., 2012a, 2012b, 2013, 2016; Teng et al., 2013, 2014, 2015, 2016), with a total of 29 *Pseudo-nitzschia* species documented so far (Furuya et al., 2018). Some of these species are known to be toxic. DA screening in *Pseudo-nitzschia* species from Malaysian waters was first conducted by Lim et al. (2010) but none was found to produce DA. Later in 2014, *P. kodamae* was

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