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First report of paralytic shellfish poisoning (PSP) caused by *Alexandrium tamiyavanichii* in Kuantan Port, Pahang, East Coast of Malaysia

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SUMMARY

Harmful algal bloom (HAB) is a proliferation of algae, which naturally produce biotoxins and cause harmful effects to humans, the environment and organisms associated with it. Paralytic shellfish poisoning (PSP) was reported for the first time in Kuantan Port, Pahang, Malaysia, in November 2013, followed by a second episode in August 2014. The toxicity level reported during the second event was as high as 3500 μg of STX equiv./100 g shellfish. Ten people were hospitalized with PSP symptoms after consuming contaminated shellfish. This study was conducted at Kuantan Port to identify the organisms responsible for these events. Water samples were collected monthly for a period of 12 months beginning in September 2014. HAB species were identified based on their morphology using light and fluorescence microscopes, and their classification was supported by molecular evidence based on internal transcribed spacer (ITS) sequences. Monthly cell abundance of *Alexandrium tamiyavanichii* was measured at four sampling stations. Toxin production by three strains isolated from the area was determined using HPLC. Our results revealed the presence of several HAB species, including the PSP-producing species *A. tamiyavanichii*. The highest cell density of *A. tamiyavanichii* was 840 cells L^{-1}. The presence of GTX components was detected in these strains. However, other toxin components could not be determined. This study reported, for the first time, the presence of PSP-producing *A. tamiyavanichii* on the Pahang coast of east Peninsular Malaysia and confirmed that the PSP events in Kuantan Port were attributable to this species. The presence of this species further indicates that several safety measures need to be considered to safeguard public health, particularly in Pahang coastal waters.

Key words: cell abundance, gonyautoxin, harmful algal bloom, shellfish.

INTRODUCTION

Harmful algal bloom (HAB) has been reported in Malaysia since 1976 (Roy 1977) and they are mainly caused by *Pyrodinium bahamense*. Since the first incident, reoccurrences of HAB caused by different species of dinoflagellates have been observed. These HAB species include *Pyrodinium bahamense var. compressum* (Roy 1977), *Cochlodinium polykrikoides* (Anton *et al*. 2008), *Alexandrium tamiyavanichii* (Usup *et al*. 2002; Kon *et al*. 2015), *Alexandrium minutum* (Lim *et al*. 2006), *Gymnodinium catenatum* (Mohammad-Noor *et al*. 2010) and *Karlodinium australe* (Lim *et al*. 2014).

Paralytic shellfish poisoning (PSP) was the first shellfish poisoning case reported in Malaysia. The first incident was recorded in Kota Kinabalu, Sabah (Fig. 1a) almost two decades ago and was caused by *P. bahamense* var. *compressum* (Roy 1977).The second PSP case was caused by *A. tamiyavanichii* in Sebatu, Malacca (Fig. 1a) (Usup *et al*. 2002; Lim *et al*. 2012). During this incident, mussels were banned from consumption, which caused losses for mussel breeders. A third case was reported in Tumpat, Kelantan (Fig. 1a) and resulted in one human fatality and six hospitalizations due to the consumption of contaminated shellfish (Lim *et al*. 2004, 2005). In this PSP incident, *A. minutum* was identified as the causative organism. To date, nine species of *Alexandrium* have been identified from Malaysian waters, including *A. tamiyavanichii*, *A. minutum*, *A. taylorii*, *A. ostenfeldii* (=*A. peruvianum*), *A. affine*, *A. leei*, *A.*cf. *tamarense*, *A. tamutum* and *A. andersonii* (Usup *et al*. 2002; Lim *et al*. 2005; Hii *et al*. 2012; Roziawati *et al*. 2016). Among those, *A. minutum* and *A. tamiyavanichii*, which have caused PSP problems in Malaysia, and other *Alexandrium* species, i.e., *A. taylorii*, *A. ostenfeldii* (=*A. peruvianum*), and *A.*cf. *tamarense*, have been shown to produce less than 1 fmol $cell^{-1}$ of PSP toxin (Lim *et al*. 2005, Lim & Ogata 2005).

Environmental factors, such as nutrient concentrations, weather conditions and physico-chemical parameters, are known to play important roles in bloom occurrences (Mohammad-Noor *et al*. 2012, 2014). High cell densities of *Pyrodinium* and *Cochlodinium* in Sabah coastal waters were positively correlated with seasonal monsoons, nutrients, physical parameters and geomorphology (Adam *et al*. 2011). An increased cell density of *Alexandrium* spp. was found in areas with high nutrient concentrations (Kon *et al*. 2015). Additionally, toxicity is also related to environmental

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