

Quantitative real-time PCR detection of a harmful unarmoured dinoflagellate, *Karlodinium australe* (Dinophyceae)

Nyuk Fong Kon,¹ Winnie L. S. Lau,² Kieng Soon Hii,^{1,2} Ing Kuo Law,² Sing Tung Teng,³ Hong Chang Lim,⁴ Kazuya Takahashi,⁵ Haifeng Gu,⁶ Po Teen Lim^{2*} and Chui Pin Leaw^{1b,2*}

¹Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak, Kota Samarahan, Malaysia,

²Institute of Ocean and Earth Sciences, University of Malaya, Bachok, Malaysia, ³Faculty of Resource Science and

Technology, Universiti Malaysia Sarawak, Kota Samarahan, Malaysia, ⁴Faculty of Applied Sciences and Computing, Tunku

Abdul Rahman University College, Segamat, Malaysia, ⁵Asian Natural Environmental Science Center, The University of

Tokyo, Tokyo, Japan and ⁶Third Oceanography Institute, Xiamen, China

SUMMARY

We investigated a harmful algal bloom (HAB) associated with the massive fish kills in Johor Strait, Malaysia, which recurred a year after the first incident in 2014. This incident has urged for the need to have a rapid and precise method in HAB monitoring. In this study, we develop a SYBR green-based real-time PCR (qPCR) to detect the culpable dinoflagellate species, *Karlodinium australe*. Species-specific qPCR primers were designed in the gene region of the second internal transcribed spacer of the ribosomal RNA gene (rDNA). The species specificity of the primers designed was evaluated by screening on the non-target species (*Karlodinium veneficum*, *Takayama* spp., and *Karenia* spp.) and no cross-detection was observed. The extractable gene copies per cell of *K. australe* determined in this study were $19\,998 \pm 505$ ($P < 0.0001$). Estimation of cell densities by qPCR in the experimental spiked samples showed high correlation with data determined microscopically ($R^2 = 0.93$). Using the qPCR assay developed in this study, we successfully detected the 2015 bloom species as *K. australe*. Single-cell PCR and rDNA sequencing from the field samples further confirmed the finding. With the sensitivity as low as five cells, the qPCR assay developed in this study could effectively and rapidly detect cells of *K. australe* in the environmental samples for monitoring purpose.

Key words: fish kill, Malaysia, ribosomal RNA gene, second internal transcribed spacer.

INTRODUCTION

Rapidly expanding aquaculture activities and industrialization in coastal regions may have contributed to an increase in nutrient loadings, and resulted in localized eutrophication. Together with favorable physical conditions, this will promote rapid growth of phytoplankton, and algal bloom is likely to occur (Hall *et al.* 2008). Some of these algal blooms are known to cause harm by producing biotoxins that can intoxicate humans or kill marine organisms. Thus, rapid harmful algal detection tools are needed for regular monitoring and early prediction of these harmful algal blooms (HABs).

Johor Strait, located between the southern Peninsular Malaysia and Singapore, is an important site of mariculture industries for both countries. Fish farms and the number of cages in the Strait have been reported to have increased compared to a decade ago, with the production of milkfish (*Chanos chanos*) at US \$ 6755/metric tons for Singapore, the second highest fish mariculture production among Southeast Asian countries in 2014 (SEAFDEC 2017). This is accompanied by the increasingly reported cases of HABs in the strait. The first HAB event was recorded in 2002, where a massive bloom of *Prorocentrum minimum* (Pavillard) Schiller occurred in the strait (Usup *et al.* 2003). In a field survey along the strait, Tan *et al.* (2013) encountered a low cell abundance of *Karlodinium* species, identified as *K. veneficum* (Ballantine) Larsen at that time based on the morphological observations. The presence of *Karlodinium* species in the strait has gained serious attention as some species from the genus are known to kill fish (e.g. Deeds *et al.* 2002; Kempton *et al.* 2002). The genus *Karlodinium* was raised from polyphyletic *Gymnodinium* sensu lato after several taxonomic amendments based on the morphological ultrastructure features, the composition of photosynthetic pigments and the nuclear-encoded large subunit ribosomal RNA gene (LSU rDNA) sequences (Daugbjerg *et al.* 2000; Bergholtz *et al.* 2005). Before 2008, the genus comprised of four species: *K. armiger* Bergholtz, Daugbjerg & Moestrup, *K. australe* de Salas, Bolch & Hallegraeff, *K. veneficum* and *K. vitiligo* (Ballantine) Larsen (Bergholtz *et al.* 2005; de Salas *et al.* 2005). Meanwhile, *K. micrum* has been synonymized as *K. veneficum* (Bergholtz *et al.* 2005). The number of described *Karlodinium* species has grown to 11, with the addition of species: *K. antarcticum* de Salas, *K. ballantinum* de Salas, *K. conicum* de Salas, *K. corrugatum* de Salas, *K. decipiens* de Salas & Laza-Martinez (de Salas *et al.* 2008), *K. corsicum* (Paulmier) Siano & Zingone, and *K. gentienii* Nézan, Chomérat & Siano (Siano *et al.* 2009). Species of *Karlodinium* have a broad global distribution and have been reported from both the Pacific

*To whom correspondence should be addressed.

Email: cpleaw@um.edu.my; ptlim@um.edu.my

Communicating Editor: Mitsunori Iwataki

Received 15 August 2016; accepted 12 April 2017.