The Application of qPCR Assays for the Early Detection of Toxic *Alexandrium* in Eastern Australian Waters

Thesis by

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Certificate of Original Authorship

I, Rendy Ruvindy hereby declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, In the Climate Change Cluster, Faculty of Science at the University of Technology Sydney.

This thesis is my own work and to the best of my knowledge it contains no materials previously published or written by another person, or substantial proportions of material which have been accepted for the award of any other degree or diploma at UTS or any other educational institution. Any contribution made to the research by others, with whom I have worked at UTS or elsewhere, is explicitly acknowledged in the thesis. I also declare that the intellectual content of this thesis is the product of my own work, except to the extent that assistance from others in the project's design and conception or in style, presentation and linguistic expression is acknowledged. This research is supported by the Australian Government Research Training Program.

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A thesis by compilation

List of Publications

- Ruvindy, R., Bolch, C.J., MacKenzie, L., Smith, K.F., Murray, S.A. (2018). *qPCR assays for the detection and quantification of multiple paralytic shellfish toxin-producing species of Alexandrium*. Frontiers in Microbiology 9:3153.
- Hallegraeff, G., Bolch, C., Condie, S., Dorantes Aranda, J.J., Murray, S., Quinlan, R., Ruvindy, R., Turnbull, A., Ugalde, S., Wilson, K. (2017). *Unprecedented Alexandrium blooms in a previously low biotoxin risk area of* Tasmania, Australia. Proceedings of the 17th International Conference on Harmful Algae 2016, 09-14 October 2016, Florianopolis, Brazil, pp.38-41.

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Abstract

Harmful algal blooms that produce Paralytic Shellfish Toxins (PSTs) are prevalent and affect shellfish harvesting areas worldwide. PSTs have caused shellfish harvesting closures and product recalls, resulting in economic losses, as well as brand damage and damage to the wider economy including the tourism industry.

In Tasmania, it is known that four PST producing species co-occur, comprising *Alexandrium catenella*, *A. pacificum*, *A. australiense* and *Gymnodinium catenatum*. In particular, of these, three species are morphologically almost identical, the species of the former *Alexandrium tamarense* species complex (*A. catenella*, *A. pacificum*, and *A. australiense*), which cannot be differentiated using light microscopy. Therefore, phytoplankton monitoring using light microscopy and total PST in shellfish using High Performance Liquid Chromatography (HPLC) may not be sufficient to allow for an early warning with enough time to take appropriate shellfish harvesting management decisions.

In this thesis, quantitative Polymerase Chain Reaction (qPCR) assays are investigated as an in-field early warning system, as well as a tool for long-term risk assessment of PST-associated harmful algal blooms. A commercial on-farm pipeline based on the collection and filtration of water samples using a custom designed gravity filter, a cell lysis, and a qPCR assay based on *sxtA4* was also developed and validated. QPCR assays based on ribosomal DNA (rDNA) 'barcoding' regions and an assay based on a gene associated with PST biosynthesis (*sxtA4*) were found to be generally specific, sensitive and efficient. The efficacy of an rDNA-based assay for cyst quantification was demonstrated, showing potential for its use as a long-term risk assessment tool for a new harvest area. However, qPCR assays based on rDNA gene regions were found to overestimate cell abundances. An analysis of rDNA copy number variation among strains of species of *Alexandrium* showed a variation of up to 3-5 orders of magnitude within a species, and was correlated significantly with genome size, which also varied within a species. An analysis of the variation in genomic copies of *sxtA4* genes showed variation as well, however this was of a lesser degree, of up to one order of magnitude. A positive correlation was found between *sxtA4* copies per cell and the total PST produced per cell, showing that the dosage effect may contribute to the regulation of PST biosynthesis.