Molecular ecology and toxicity of *Alexandrium pacificum* in Australian waters

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This thesis has been submitted as the partial fulfillment of the requirements for the degree of Doctor of Philosophy

Certificate of Original Authorship

I, Abanti Barua declare that this thesis, is submitted in fulfilment of the requirements for the award of PhD, in the School of Life Sciences/ Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

This research is supported by the Australian Government Research Training Program.

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Thesis Format

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List of Publications

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Barua, A., Ajani, P., Farrell, H., Zammit, A., Brett, S., Hill, D., Murray, S., 2020. Case study 2: Alexandrium pacificum in Twofold Bay. In Farrell H, Ajani P, Murray S, Baker P, Webster G, Brett S, Zammit A, Barua A, Hill D. (2020) Time series of harmful algal blooms in New South Wales. In Richardson A.J, Eriksen R, Moltmann T, Hodgson-Johnston I, Wallis J.R. (Eds). State and Trends of Australia's Ocean Report. doi: 10.26198/5e16acea49e84

Verma, A., Barua, A., Ruvindy, R., Savela, H., Ajani, P.A. and Murray, S.A., 2019. The genetic basis of toxin biosynthesis in dinoflagellates. Microorganisms, 7(8), p.222.

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Thesis Abstract

Species of the genus *Alexandrium* are one of the most studied dinoflagellates due to their production of the neurotoxins, Paralytic Shellfish Toxins (PSTs). PST-associated Harmful Algal Blooms (HABs) appear to be increasing around the world. The appearance of species of *Alexandrium* is now frequent in coastal waters of Australia, particularly in Tasmania and New South Wales. The East Australian Current (EAC) flows southward along the coasts of eastern Australia and has been reported as a global 'climate change hotspot'. Despite such potent neurotoxin production, the ecology, toxicity and population dynamics of *Alexandrium* species are little known in Australia.

In this thesis, I have investigated the first record of PST above the regulatory limit of 0.8 mg/kg produced by *Alexandrium pacificum* in the commercial aquaculture area of south-eastern Australia. During this unprecedented event, the maximum reported PST concentration in mussel tissue was 7.2 mg/kg STX equivalent. A comparative differential gene expression study was conducted to understand the gene regulation of PST related genes in *Alexandrium pacificum*. In this study, experiments were performed in the presence and absence of the copepodamide-synthesizing copepod *Parvocalanus crassirostris.* Using Nanostring gene technology, results identified the up-regulation of the key PST-related gene *sxtA,* in particular, one paralogue each of domains of *sxtA1* and *sxtA4*. An increased rate of PST production in the two PST-producing strains in the presence of copepods was identified, however it did not influence gene related transcript abundance. This indicated that post-transcriptional regulation processes may be important in regulating PST production in *Alexandrium pacificum*. In this thesis, the population structure of *Alexandrium pacificum* was examined in different Australia boundaries currents – the East Australian Current (EAC) and the Leeuwin Current (LC). This study was conducted using Single Nucleotide Polymorphisms (SNPs) as genetic markers and represents the first time the population structure of a phytoplankton species has been examined in Australian waters. Strains from South Australia and Western Australia clustered as a group, and were separated from the strains isolated from the EAC region, indicating the presence of genetic isolation of *A. pacificum* strains in Australian waters. It suggests that *A. pacificum* is more likely to represent a long resident population, and it is not a recent bioinvasion in Western Australian waters.

The results identified during this study significantly advance the understanding of *Alexandrium,* especially their abundance, diversity, population structure and the regulation of the PST-related genes.