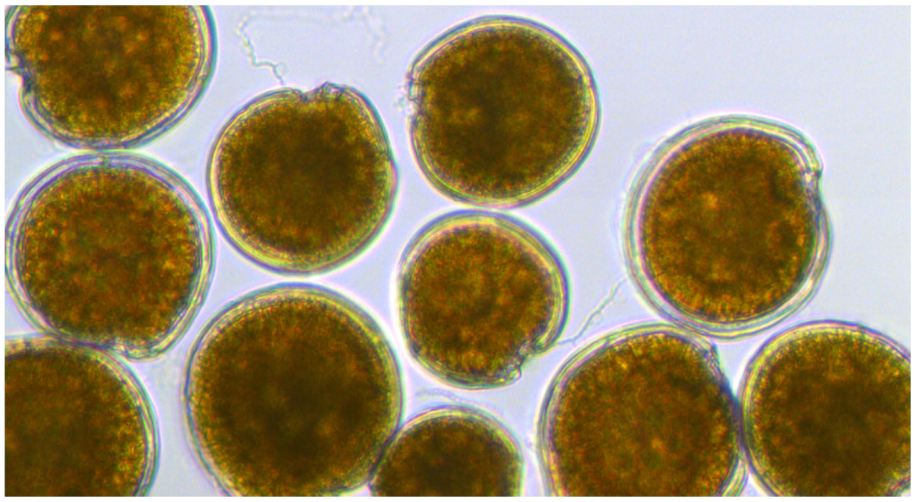
Toxicology and Ecology of *Gambierdiscus* from Australia: A Dinoflagellate Genus Associated with Ciguatera Fish Poisoning



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B.Sc. (Hons)

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for the degree of Doctor of Philosophy in Science.

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Climate Change Cluster (C_3)
University of Technology Sydney

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Certificate of Authorship/Originality

I, Michaela E. Larsson declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced 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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Publications and Research Outputs

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Larsson, M. E., Smith, K. F., Doblin, M. A. First description of the environmental niche of the dinoflagellate species *Coolia palmyrensis*, *C. malayensi*s and *C. tropicalis* (Dinophyceae) from eastern Australia. (Journal of Phycology; in press).

Larsson, M. E., Laczka, O. F, Harwood, D. T., Lewis, R. J., Himaya, S. W. A., Murray, S. A., Doblin, M. A. (2018) Toxicology of *Gambierdiscus* (Dinophyceae) from tropical and temperate Australian waters. Marine Drugs. 16(1):7, doi:10.3390/md16010007.

Larsson, M. E., Harwood, D. T, Lewis, R. J., Himaya, S. W. A., Doblin, M. A. (2018). Toxicological characterisation of *Fukuyoa paulensis* (Dinophyceae) from temperate Australia. Phycological Research. 67(1):65-71, doi: 10.1111/pre.12349

Larsson, M. E., Laczka, O. F., Suthers, I. M., Ajani, P. A., Doblin, M. A. (2018) Hitchhiking in the East Australian Current – Rafting as a dispersal mechanism for harmful epibenthic dinoflagellates. Marine Ecology Progress Series, 59:49-60, doi: 10.3354/meps12579.

Munday, R., Murray, S., Rhodes, L. L., Larsson, M. E., Harwood, D. T. (2017). Ciguatoxins and maitotoxins in extracts of sixteen *Gambierdiscus* isolates and one *Fukuyoa* isolate from the South Pacific and their toxicity to Mice by intraperitoneal and oral administration. Marine Drugs 15(7):208, doi: 10.339/md15070208.

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Kretzschmar, A. L. and Larsson, M. E., Hoppenrath, M., Doblin, M. A., Murray, S. A. (2018). Characterisation of *Gambierdiscus lewisii* sp. nov. and *Gambierdiscus holmesii* sp. nov. from the Great Barrier Reef, Australia. Poster presentation. 18th International Conference on Harmful Algae (ICHA), Nantes, France.

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Larsson, M. E., Bush, S. A., Baker, K. G., Murray, S. A., Doblin, M. A. (2016). Mechanisms for temperate range extension of *Gambierdiscus*, the causative organism of the human illness Ciguatera Fish Poisoning. Oral presentation. 17th International Conference on Harmful Algae. Florianopolis, Brazil.

Larsson, M. E., Baker K. G., Laczka, O. F., Samardzic, K., Rodgers, K. J., Harwood, T. D., Murray, S. A., Doblin, M. A. (2015). Understanding the ecological niche of toxin producing epibenthic dinoflagellates. Oral presentation. 29th Australasian Society for Phycology and Aquatic Botany Conference, Hobart, Australia. Awarded: Best Student Presentation.

Other outputs arising directly from this doctoral thesis

Australia's Science Channel (2018). Marine microalgae 'rafting' into new territory – research inspired by famous Kon-Tiki expedition. https://australiascience.tv/marine-microalgae-rafting-into-new-territory/.

Larsson, M. E. (2018). Ciguatera Fish Poisoning in Australia: investigating the causative organism. ECSA Bulletin. (in press).

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University of Technology Sydney (UTS) News Room. (2016). Microalgal research reaps rewards. https://www.uts.edu.au/research-and-teaching/our-research/climate-change-cluster/news/microalgal-toxin-research-reaps.

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Abbreviations

ANOVA	Analysis of Variance
ASP	Amnesic Shellfish Poisoning
aq	Aqueous
AZP	Azaspiracid Shellfish Poisoning
BHAB	Benthic Harmful Algal Bloom
bp	Base Pair
BSA	Bovine Serum Albumin
CFP	Ciguatera Fish Poisoning
CTX	Ciguatoxin
CO_2	Carbon Dioxide
DNA	Deoxyribonucleic Acid
DSP	Diarrhetic Shellfish Poisoning
EAC	East Australian Current
FBS	Foetal Bovine Serum
FLIPR	Fluorescent Imaging Plate Reader
F_v/F_m	Maximum quantum yield of photosystem II
GTR	General Time Reversible
GTR+G	General Time Reversible with Gamma-shaped among site variation
HA	Haemolytic Assay

HAB	Harmful Algal Bloom
HPLC	High Performance Liquid Chromatography
LC-MS/MS	Liquid Chromatography Tandem-Mass Spectrometry
LD	Lethal Dose
LSU	Large ribosomal subunit gene
MBA	Mouse Bioassay
nMDS	Non-metric Multidimensional Scaling
MTX	Maitotoxin
NSP	Neurotoxic Shellfish Poisoning
N2a	Mouse Neuroblastoma Assay
PCO	Principle Coordinate Analysis
PCR	Polymerase chain reaction
PERMANOVA	Permutational Analysis of Variance
PSP	Paralytic Shellfish Poisoning
RBA	Receptor Binding Assay
RPMI	Roswell Park Memorial Institute medium
SH-SY5Y	Human Neuronal Cell Line
sp.	Species (singular)
spp.	Species (plural)
TFA	Trifluoroacteic Acid
UPLC	Ultra Performance Liquid Chromatography

Abstract

Some species from the epibenthic marine microalgal genus *Gambierdiscus* produce potent neurotoxins, such as ciguatoxins (CTXs) and maitotoxins (MTXs), which can accumulate in the marine food web and cause the human illness Ciguatera Fish Poisoning (CFP). The genus typically has a tropical distribution and is known to occur in the Great Barrier Reef region of north east Australia, although recently, populations have been documented in more temperate locations. In this thesis, a toxicological and ecological approach was used to investigate CFP causing organisms in Australia, with an emphasis on assessing the potential for temperate range extension of the genus in this region. Monoclonal isolates of *Gambierdiscus* were established from a tropical and a temperate location in eastern Australia and formed the foundation of experimental work. Four species of *Gambierdiscus* (*G.* cf. *pacificus, G.* cf. *silvae, G. carpenteri and G. lapillus*) were identified from the tropical location and only *G. carpenteri* was identified at the temperate location. Liquid Chromatography Tandem-Mass Spectrometry (LC-MS/MS) was used to assess whether isolates produced known microalgal CTXs (P-CTX-3B, 3C, 4A and 4B) and MTX-1, but these characterised toxins were not detected in any of the *Gambierdiscus* strains. Putative MTX-3, however, was detected in all strains, except the temperate *G. carpenteri* isolates. Using the novel Ca^{2+} influx bioassay, compounds displaying CTX-like activity were identified in extracts of *G.* cf. *pacificus, G.* cf. *silvae* and *G. lapillus*, and compounds displaying MTX-like activity were detected in all species tested. Fitness curves across environmental gradients of temperature, salinity and irradiance showed *Gambierdiscus* species can grow across a broad range of environmental conditions. The environmental niche of the tropical strains was not

significantly different from that of the temperate strains, suggesting that tropical toxin producing *Gambierdiscus* species also have the capacity to occupy temperate locations. Rafting on detached macrophyte fragments that are transported poleward in the East Australian Current was identified as the likely natural long-distance dispersal mechanism for *Gambierdiscus* species in eastern Australia. The ability of *Gambierdiscus* to colonise new temperate locations was examined by studying the growth of different strains co-cultured within both natural and artificial epibenthic microalgal communities. These experiments confirmed that it may only require a single pulse of very few cells for successful colonisation of *Gambierdiscus*. This thesis advances knowledge about the diversity and toxicology of *Gambierdiscus* in eastern Australia, identifies the potential cause of CFP from this region and provides experimental evidence of the mechanisms that could facilitate temperate range extension of the genus. Results from this thesis therefore provide fundamental information for developing a management strategy to mitigate the risk of human exposure to CFP in eastern Australia.