# Development and novel application of an *in vitro* fish gill cell assay to elucidate the ichthyotoxic mechanism of the microalga *Chattonella marina* (Raphidophyceae)

by

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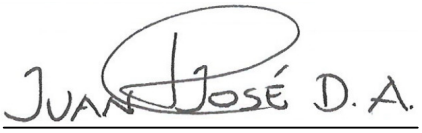
I declare that the material presented in this thesis is original, except where due acknowledgement is given, and has not been accepted for the award of any other degree or diploma



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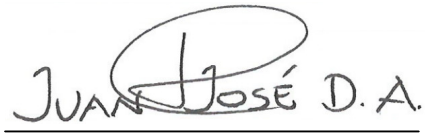

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T. David Waite, Aurélie Godrant and Andrew L. Rose contributed to the idea and presentation

César D. Tovar and Gregory M. Woods contributed with training for part of the experimental work

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T. David Waite contributed with training for part of the experimental work and discussion

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# Dedication

To my parents,
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for their love, support, patience and guidance in life

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# Contents

# Abstract

*Chattonella marina* algal blooms have been associated with major farmed and wild fish mortalities in tropical (Mexico), subtropical (South Australia) and temperate regions (Japan). However, the precise toxic mechanisms involved remain incompletely known and disputed. A novel sensitive *in vitro* assay using the rainbow trout cell line RTgill-W1 was developed to assess toxicity of lipid extracts, ruptured cells and intact cultures of 6 strains of *C. marina. Chattonella* was found to be less toxic than the haptophyte *Prymnesium parvum*, but more toxic than the raphidophytes *Heterosigma akashiwo, Fibrocapsa japonica* and dinoflagellate *Karenia mikimotoi*. Ruptured cells from Australian CMPL01 and Japanese N-118 *C. marina* strains were the most toxic, decreasing gill cell viability by 71 and 65%, respectively. The Mexican CMCV-1 strain was the least toxic (≤35%), possibly because it is larger and less fragile than other strains. *Chattonella marina* is unique among ichthyotoxic microalgae in its high production of superoxide anion (≤19 pmol $cell^{-1}$ $hr^{-1}$). Sonicated cultures showed higher levels of superoxide than intact cultures (19.0 vs 9.5 pmol $cell^{-1}$ $hr^{-1}$, respectively). However, $O_2^-$ on its own did not appear to be the main cause of toxicity (only 14% loss of cell viability). Superoxide production was highest when grown in medium enriched with 1 µM Fe(III) compared to the 5 µM standard medium (25 vs 9.5 pmol $cell^{-1}$ $hr^{-1}$), especially after a sudden change from dark adaptation to light conditions (200 µmol photons $m^{-2}$ $s^{-1}$) (up to 37.6), but lowest (4.1) at 100 nM Fe(III). The three major fatty acids in *C. marina* were palmitic (PA), eicosapentaenoic (EPA), and octadecatetraenoic (OTA) acids. Higher levels of EPA were found in ruptured cells (21.4 – 29.4% of total fatty acids) compared to intact cells (8.5 – 25.3%). Gill damaging effects from free fatty acid (FFA) fractions were conclusively demonstrated ($LC_{50}$ at 1 hr of 0.44 µg $mL^{-1}$), and remain candidates of ichthyotoxicity when well-defined toxins such as brevetoxin or karlotoxin can be ruled out as causative factors. The aldehydes 2*E*,4*E*-decadienal and 2*E*,4*E*-heptadienal, which may be generated by FFA oxidation, also showed high impacts on gill cell viability, with $LC_{50}$ (at 1 hr) of 0.34 and 0.36 µg $mL^{-1}$, respectively. In conclusion, *C. marina* was more toxic after cell disruption and when switching from dark to light conditions, associated with higher EPA levels and a

higher production of superoxide anion. Implications of this work for finfish aquaculture management and mitigation are discussed.