Diarrhetic Shellfish Toxins in Tasmanian coastal waters: causative dinoflagellate organisms, dissolved toxins and shellfish depuration

by

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Image of Freycinet Marine Farm taken from website www.wineglassbay.com. Insets from left to right: diving at Sullivans Cove; SPATT bags; and micrographs of *Dinophysis acuminata* (left) and *D. fortii* (right).
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Abstract

The Diarrhetic Shellfish Toxins (DST), okadaic acid (OA) + dinophysistoxin-1 (DTX-1), were detected above the regulatory limit of 0.20 µg/g of digestive gland (DG) in (non-commercial) blue mussels (Mytilus edulis) from Sullivans Cove, Tasmania. Pectenotoxin-2 (PTX-2), PTX-2 seco acids and 7-epi-PTX-2 SA were also detected in mussels. This was associated with the occurrence of the toxic dinoflagellates, Dinophysis acuminata and D. fortii, which were seasonally prevalent at high cell densities (up to 7,380 cells/L for D. acuminata, 500 cells/L for D. fortii). A high density of D. truncata (1,850 cells/L) did not result in increased DST levels in M. edulis at Parsons Bay, Tasmania, suggesting that this may be a non- or weakly toxic dinoflagellate.

Subtle variations among Dinophysis morphotypes can pose problems for rapid and accurate identification. Tasmanian sequences of the D1-D3 region of the large subunit rDNA of D. fortii were indistinguishable from those of D. fortii from France and D. acuta from the North Atlantic, while Tasmanian D. acuminata was indistinguishable from European and New Zealand D. acuminata. Genetic sequencing of New Zealand D. acuta failed to discriminate between Tasmanian D. fortii and New Zealand D. acuta and neither did sequencing discriminate between European D. fortii and D. acuta.

A field depuration experiment was conducted in the Derwent River by placing M. edulis in 38 µm mesh size cages to screen out Dinophysis plankton cells. Mussels displayed biphasic depuration kinetics with a faster rate of PTX loss over the first 30 days followed by an increase of OA + DTX-1 depuration once there was no further change in PTX levels. The slow rate of depuration of OA + DTX-1 from day 15 to 30 followed by an increase in depuration may be attributed to mussels using lipid storage during a period of reduced food availability leading to a release of toxins in bound fractions. Solid Phase Adsorption Toxin Tracking (SPATT) detected dissolved DST in the Derwent River seawater medium at levels as high as 0.34 OA + DTX-1 µg/SPATT bag.
Cellular and exuded toxicity of *Prorocentrum lima* varied between two culture strains isolated from different locations in Tasmania, Australia. Cellular OA was greater in the Little Swanport (PLLSP) strain (36 pg/cell) compared to the Louisville Point (PLLV) strain (3.8 pg/cell), which was the only strain producing DTX-1. PTX-2 was produced by both strains at small concentrations up to 1.2 pg/cell. This is the first reported occurrence of PTX-2 produced by *P. lima*. The Louisville strain excreted higher concentrations of OA (reaching 18 µg/SPATT bag) in the first 20 days compared to the Little Swanport strain (11 µg OA/SPATT bag). For both strains this declined to 4 µg/SPATT bag on day 40. Both strains exuded higher dissolved toxin levels at low cell abundance of 1,200 cells/L (PLLV strain reaching 1.6 µg OA + DTX-1/SPATT bag) compared to at 2,400 cells/L (0.4 µg OA + DTX-1/SPATT bag). Tasmanian strains of *P. lima* were more toxic than other global strains and poses a potential DSP risk to Tasmanian shellfish farms.

*In-vitro* experiments with *Prorocentrum lima* suggest that dissolved toxins are exuded from DST producing dinoflagellates as well as from depurating mussels. Most of the DST was present dissolved in the seawater (94 %) when SPATT bags were exposed to *P. lima* cultures (6 % of DST in cells). Only a small amount of DST (1 %) was detected in the seawater medium when SPATT bags were exposed to contaminated mussels (99 % of DST in mussels). OA displayed an increase by more than 0.11 µg/g DG in mussels immersed in dissolved DST for 48 hrs indicating that mussels can accumulate DST in *in-vitro* conditions.

Dissolved DST can pose an additional threat to shellfish farms and can extend harvest closure periods after toxic dinoflagellate blooms. Toxicity differences among dinoflagellate species and strains can pose problems for shellfish monitoring programs and may require phytoplankton regulatory limits to be varied according to locality.
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- *P. lima* cultures (PLLSP and PLLV) were obtained from the aquatic botany culture facility at the University of Tasmania. *P. lima* cultures were established by Imogen Pearce in 2000. *Thalassiosira weissflogii* (CCMP1336 strain) was obtained from the CSIRO culture facility.

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Table of Contents

Chapter 1: General Introduction................................................................................. 1
  Definition of DST .................................................................................................. 2
  Global Distribution ............................................................................................... 3
  Analysis of DST .................................................................................................... 4
  Monitoring DST in Australia ................................................................................. 6
  Aims of thesis ...................................................................................................... 12
  References ........................................................................................................... 13

Chapter 2: Diarrhetic Shellfish Toxins in Tasmanian shellfish and their causative
  dinoflagellate organisms ......................................................................................... 19
  Abstract ............................................................................................................... 20
  2. 1.  Introduction................................................................................................. 20
  2. 2.  Material and Methods.................................................................................. 21
      2. 2. 1.  Study Site ............................................................................................ 21
      2. 2. 2.  Sample collection ................................................................................ 22
          2. 2. 2. 1.  Phytoplankton .............................................................................. 22
          2. 2. 2. 2.  Mussels ........................................................................................ 22
      2. 2. 3.  Toxin extraction from Mytilus edulis and phytoplankton ............... 23
      2. 2. 4.  HPLC/MS analysis of toxins ............................................................... 24
      2. 2. 5.  Statistical Analyses .............................................................................. 25
  2. 3.  Results ........................................................................................................ 25
      2. 3. 1.  Sullivans Cove mussel toxin content .................................................... 25
      2. 3. 2.  Parsons Bay mussel toxin content ........................................................ 26
      2. 3. 3.  Sullivans Cove phytoplankton toxin content .......................................... 26
  2. 4.  Discussion ................................................................................................... 30
      2. 4. 1.  Sullivans Cove mussel toxin content .................................................... 30
      2. 4. 2.  Parsons Bay mussel toxin content ........................................................ 36
      2. 4. 3.  Sullivan Cove phytoplankton toxin content .......................................... 37
  2. 5.  Conclusion .................................................................................................. 39
      References ........................................................................................................ 41

Chapter 3: Morpholotaxonomy and Genetic affinities of Tasmanian D. forti and D.
  acuminata ............................................................................................................. 51
Chapter 1: Isolation and Identification of Dinophysis Species in Tasmanian Waters

Abstract ............................................................................................................... 52

1. Introduction ...................................................................................................... 52

2. Material and Methods ..................................................................................... 53
   2.1. Study Site and phytoplankton sample collection .................................. 53
   2.2. PCR amplification and cycle sequencing ............................................. 54
   2.3. Sequence alignment and phylogenetic analysis .................................... 54

3. Results ............................................................................................................ 54
   3.1. Morphology of Dinophysis species ...................................................... 54
   3.2. Sequencing of the LSU gene ................................................................ 57

4. Discussion ....................................................................................................... 58

5. Conclusion ..................................................................................................... 61

References ........................................................................................................... 62

Chapter 2: Diarrhetic Shellfish Toxin Accumulation and Depuration in Tasmanian Mussels (Mytilus edulis)

Abstract ............................................................................................................... 65

1. Introduction ...................................................................................................... 66

2. Methods .......................................................................................................... 67
   2.1. Field Depuration experiment ............................................................... 67
   2.2. Toxin extraction from Mytilus edulis ................................................... 68
   2.3. HPLC/MS analysis of toxins ............................................................... 69
   2.4. Phytoplankton collection and enumeration ........................................... 70
   2.5. Statistical Analyses .............................................................................. 70

3. Results ............................................................................................................ 71
   3.1. OA + DTX-1 ....................................................................................... 71
   3.2. PTX-2 and PTX derivatives ................................................................. 71

4. Discussion ....................................................................................................... 75

5. Conclusion ..................................................................................................... 78

References ........................................................................................................... 79

Chapter 3: Can Mussels (Mytilus edulis) Take up Dissolved DST? Dinophysis Field and
Prorocentrum lima Laboratory Experiments Using Solid Phase Adsorption Toxin Tracking (SPATT)

Abstract ............................................................................................................... 81

1. Introduction ...................................................................................................... 82

2. Methods and Materials .................................................................................. 84

3. Results ............................................................................................................ 86
   3.1. OA + DTX-1 ....................................................................................... 86
   3.2. PTX-2 and PTX derivatives ................................................................. 86

4. Discussion ....................................................................................................... 89

5. Conclusion ..................................................................................................... 92

References ........................................................................................................... 93
5. 2. 1. Study site ......................................................................................................... 84
5. 2. 2. Phytoplankton collection and enumeration .............................................. 84
5. 2. 3. Mussel collection ....................................................................................... 85
5. 2. 4. Solid Phase Adsorption Toxins Tracking (SPATT) ........................................ 85
5. 2. 5. Laboratory experiments ............................................................................. 86
  5. 2. 5. 1. Dissolved DST from Prorocentrum culture ......................................... 86
  5. 2. 5. 2. Mussels and T. weissflogii exposed to dissolved DSP toxins ........... 87
5. 2. 6. Toxin extraction from Mytilus edulis, phytoplankton and SPATT bags .... 88
5. 2. 7. HPLC/MS analysis of toxins ....................................................................... 90
5. 3. Results .............................................................................................................. 91
  5. 3. 1. Sullivans Cove Solid Phase Adsorption Toxin Tracking (SPATT) .......... 91
  5. 3. 2. P. lima cultures ....................................................................................... 91
  5. 3. 3. Dissolved DST uptake by laboratory mussels ......................................... 94
5. 4. Discussion ...................................................................................................... 97
  5. 4. 1. Field Study ............................................................................................. 97
  5. 4. 2. P. lima laboratory experiments ............................................................... 100
  5. 4. 3. Mussels exposed to dissolved DSP toxins ............................................. 102
  5. 4. 3. Effect of food availability on mussel toxins ........................................... 104
5. 5. Conclusion ................................................................................................... 105
References ............................................................................................................. 107
Chapter 6: Conclusions .......................................................................................... 112
  DST in Tasmanian Dinophysis and mussels ....................................................... 113
  Dinophysis morphotaxonomy and phylogenetics ............................................. 114
  Accumulation and depuration kinetics ............................................................. 114
  P. lima cellular and dissolved DST ................................................................. 115
Future Work ......................................................................................................... 117
References ............................................................................................................. 118
Appendix ............................................................................................................... 121