Prymnesiophytes of New Zealand's coastal waters: taxonomy, physiology and ecology.

A thesis presented in partial fulfilment of the requirements
for the degree
of Doctor of Philosophy
in Plant Biology and Biotechnology at

Massey University

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1994

Abstract

Prymnesiophytes are an important component of the marine phytoplankton of New Zealand coastal waters, but there is little knowledge of the taxonomy, physiology or ecology of local strains. The Class comprises four orders which contain putative families and genera of the microscopic organisms, the microalgae. The Prymnesiophytes are known for their ability to explode in population numbers into "blooms"; one of New Zealand's most common bloom-formers is the coccolithophore *Emiliania huxleyi* (Prymnesiophyceae), which dominated the extensive phytoplankton blooms observed around New Zealand's coastline during 1993 - 94. Aspects of the physiology of and factors contributing to bloom formation in *E.huxleyi* are investigated.

The phenomenon of seasonal blooms, a common occurrence amongst the microalgae, is reviewed. Studies of the blooms which occurred along the north-east coastline of New Zealand in 1992-93 showed that these were unusual events that were linked to the climatic conditions at that time, in particular cold sea temperatures associated with an "El-Niño" phase of the Southern Oscillation Index in the southern Pacific Ocean. The dominant microalgae were *Gephyrocapsa oceanica* (Prymnesiophyceae) and *Fibrocapsa japonica* (Raphidophyceae) and it is probable that the conditioning of the coastal waters by these microalgae had a role in the succeeding toxic event, in which human illnesses were definitively linked to shellfish toxins due to microalgae for the first time in New Zealand.

Allelopathic, or chemical, interactions between microalgae were investigated in this study and *Prymnesium parvum* and *P.patellifera* caused inhibition of the growth of species from several microalgal classes. Unfortunately *P.patellifera* rapidly lost its inhibitory activity *in vitro*, but *P.parvum* remained active for

several years in culture and was therefore selected as a positive control for the bioassays for ichthyotoxicity that were developed.

The class Prymnesiophyceae includes toxic species of three genera, namely *Chrysochromulina, Prymnesium* and *Phaeocystis.* Ichthyotoxin bioassays based on toxin sensitive microalgae (*Chattonella antiqua* and *Heterocapsa triquetra*), shellfish larvae (*Haliotis iris*), brine shrimps (*Artemia salina*) and salmon erythrocytes (*Oncorhynchus tshawytscha*), were developed or refined and evaluated to enable the rapid and inexpensive detection of the haemolytic and cytolytic prymnesiophyte toxin, prymnesin.

The novel quadriflagellate species *C.quadrikonta,* which bloomed in north-east New Zealand, May 1994, exhibited low levels of haemolytic activity in stationary phase cultures grown in standard nutrient medium (at 18^0C, 100 μmol $m^{-2}s^{-1}$) as determined by the erythrocyte assay; no other *Chrysochromulina* species tested was toxic.

Six locally occurring species were identified by electron microscopic examination of scales and light and electron microscopic observation of cultured isolates in this study. Seventeen of the nearly fifty named species of *Chrysochromulina* have now been identified in New Zealand. The average cell sizes and unmineralised spine scale lengths of the New Zealand isolates of *C.ericina, C.hirta* and *C.quadrikonta* were slightly larger than for their type species and the calcareous scales of *E.huxleyi* and *G.oceanica* were more heavily calcified than their northern hemisphere counterparts. No gradation of calcification with increased latitude was observed for the coccolithophores, as had been noted previously, and this might reflect the consistently lower sea temperatures prevailing, due to the unusually protracted "El-Niño" climatic conditions.

Fluorescent probes proved to be useful tools for the differentiation of some morphologically-like species under the light microscope: Calcofluor white helped distinguish between cells of *C.ericina* and *C.quadrikonta.* The differentiation of the genera *Prymnesium* and *Chrysochromulina* was enabled through the specific binding of fluorescently-tagged wheat germ lectin to *Chrysochromulina* species.

The growth characteristics and cultural idiosyncrasies of several southern hemisphere *Chrysochromulina* isolates have been described and compared with the toxic northern hemisphere relative, *C.polylepis*. *C.polylepis, C.ericina* and *C.hirta* fell into a temperate group on the basis of optimum growth rates (doublings d^{-1}), while *C.acantha, C.apheles, C.camella* and *C.quadrikonta* fell into a sub-tropical group. All but *C.acantha* grew equally well with potassium nitrate, urea or ammonium chloride; *C.acantha* grew significantly slower with urea as nitrogen source. Only *C.quadrikonta* had a selenium requirement for growth. Maximum growth rates (doublings d^{-1}) recorded *in vitro* were *C.acantha*, 1.2; *C.apheles*, 0.9; *C.camella*, 1.1; *C.ericina*, 1.5; *C.hirta*, 2.4 and *C.quadrikonta*, 1.4. The *Chrysochromulina* species and *E.huxleyi* and *G.oceanica* grew at low light intensities (25 μmol $m^{-2}s^{-1}$), which could give these prymnesiophytes a competitive advantage in bloom situations, where shading due to the phytoplankton biomass can occur.

The New Zealand isolate of *G.oceanica* grew optimally at salinities of 17 - 29º/oo, pH of 8.4 - 8.9 and temperatures of 20 - 25ºC; *E.huxleyi* grew optimally at 29º/oo, pH7.5 - 8.9 and 15 - 25ºC. *G.oceanica* grew equally well with ammonium chloride, urea or nitrate as nitrogen source; *E.huxleyi* grew optimally with ammonium chloride. Maximum growth rates recorded were 1.9 doublings d^{-1} for *E.huxleyi* and 1.4 doublings d^{-1} for *G.oceanica*.

Acknowledgments

Special thanks are due to David Fountain, Charley O'Kelly, Julie Hall and Don Grant. I have appreciated their rigorous supervision and positive criticism, which have always been carried out in a thoughtful and constructive way.

Thanks to my colleagues at Cawthron Institute, Nelson, for their contributions to my work: Henry Kaspar and Doug Mountfort have given excellent feed-back on publication and thesis drafts and the support of Graeme Robertson, CEO at Cawthron Institute, has been vital and always forthcoming. Lincoln MacKenzie collected the first isolate of *Chrysochromulina quadrikonta* and has consistently shared information with me and Brendon Burke provided the first isolate of *C.ericina.* I have enjoyed collaborating with Allison Haywood on the studies of Northland and Hauraki Gulf blooms and value her enthusiasm. David White carried out the acrylic acid analyses, Doug Mountfort rendered *Heterocapsa triquetra* axenic for the bioassay experiments and Brendon Burke, Rod Asher, Maggie Atkinson and Aaron Quarterman have contributed at various times and in various ways to the thesis with excellent technical support. In particular Brendon has put time and effort into setting up a bank of maps, which I have incorporated into this thesis. I have also appreciated the support of John Stark and John Hayes with computing and statistical problems.

The consistent and excellent assistance of Doug Hopcroft and Raymond Bennett, Hort Research, Palmerston North, New Zealand with electron microscopy is acknowledged and also the assistance of Rob Thomson and Teeba Lundy at Victoria University's EM Unit.

I wish to acknowledge Isao Inouye and Masanobu Kawachi, University of Tsukuba, Ibaraki, Japan, for making available their unpublished results on the Japanese strain of *C.quadrikonta* and Tony Edwards, Stratigraphic Solutions, Wellington, New Zealand for sharing his coccolithophorid expertise.

Assistance with obtaining fish erythrocytes was willingly given by Regal Salmon Ltd., Blenheim; Southern Ocean Seafoods, Rai Valley; Colin Anderson, Wallaceville Animal Research Centre, Upper Hutt and Jan de Zwart, MAF Regulatory Authority, Blenheim. Thanks also to Jan Holland, Crop and Food Research, Nelson.

Several microalgal species were provided by Len Tong, MAF Fisheries, Mahanga Bay hatchery, Wellington and *Prymnesium* species by Hoe Chang, Oceanographic Institute, NIWA, Wellington. Julie Hall, Ecosystems, NIWA, Hamilton, provided the fluorescently labelled beads for the phagotrophy experiments.

Collaboration with Paul Lupi, New Zealand Marine Farmers Association, Blenheim, Peter Smith and Brian Jones, MAF Fisheries, Wellington, Greg Sloane and Bill Trusewich, MAF Regulatory Authority, Wellington, Bill Ballantine, University of Auckland, Barbara Hickey, Auckland Regional Council, Simon Marwick, Big Glory Seafoods, Stewart Island, Barry Peake, University of Otago, Dunedin, Tony Beauchamp, Northland Area Health Services and Maurice Miles, Waitemata Health Services, has been valuable and enjoyable.

A New Zealand Universities Postgraduate Scholarship, a Cawthron Scholarship, Massey University Grants Committee funding and contracts with the Foundation for Research, Science and Technology have been appreciated. The New Zealand Salmon Farmers Association and the New Zealand Marine Sciences Society also provided funds for travel to overseas conferences.

Preface

The following papers were submitted for publication during the course of this study. Copies of the published papers are found inside the back cover of the thesis:

❶ Rhodes,L.L., Haywood,A.J., Ballantine,W.J., MacKenzie,A.L. (1993) Algal blooms and climate anomalies in north-east New Zealand, August to December, 1992. *New Zealand journal of marine and freshwater research* **27**, 419-430.

❷ Rhodes,L.L., O'Kelly,C.J., Hall,J.A. (1994) Comparison of growth characteristics of New Zealand isolates of the prymnesiophytes *Chrysochromulina quadrikonta* and *C.camella* with those of the ichthyotoxic species *C.polylepis. Journal of plankton research* **16**, 69-82.

❸ Jones,J.B. and Rhodes,L.L. (1994) Suffocation of pilchards . (*Sardinops sagax*) by a green microalgal bloom, Wellington Harbour, New Zealand, December 1993. *New Zealand journal of marine and freshwater research*, **28**, 379-384.

❹ Rhodes,L.L., Edwards,A.R., Peake,B.M., MacKenzie,A.L., Marwick,S. (1994) Bloom and growth characteristics of the coccolithophores *Gephyrocapsa oceanica* and *Emiliania huxleyi* (Prymnesiophyceae) in New Zealand's coastal waters. *New Zealand journal of marine and freshwater research* (submitted November 1994).

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Abbreviations

aff.	has an affinity with
BSA	bovine serum albumin
cf.	compares with
CHRY	*Chrysochromulina*
ConA	Concanavalin A
d	day
DAPI	4'6-diamidino-2-phenylindole
DMS	dimethylsulphide
DTT	dithiothreitol
ECA	*Erythrina cristagalli* A (coral tree) lectin
EDTA	ethylenediamine tetraacetic acid
FITC	Fluorescein isothiocyanate
GC	gas chromatography
GP	general purpose
GR	growth rate
h	hour
HPA	*Helix pomatia* A (snail) lectin
HPLC	high performance liquid chromatography
MAF ORPP	Ministry of Agriculture and Fisheries operational research phytoplankton programme
N:P	nitrogen:phosphate
Na; NaOH	sodium; sodium hydroxide
PAHBAH	para hydroxy-benzoic acid hydrazide
PEA	*Pisum sativum* (pea) lectin
PHA	*Phaseolus limensis* (lima bean) lectin
PWM	*Phytolacca americana* (pokeweed) lectin
SBA	*Glycine max* (soy bean) lectin
Se	selenium
SEM	scanning electron microscopy
sp.	species
SST	sea surface temperature
tD_{50}	time until 50% of bioassay organisms are dead
tM_{50}	time until 50% of bioassay organisms are morbid
TEM	transmission electron microscopy
TES	(N-tris[hydroxymethyl]-methyl-2-aminoethane-sulfonic acid)
UEA	*Ulex europaeus* (gorse) lectin
UV	ultraviolet
v/v	volume per volume
WGA	*Triticum vulgaris* (wheat germ) lectin
$^{0}/_{00}$	salinity (grams salt per kilogram seawater) in parts per thousand