Investigation of the Toxicology and Public Health Aspects of the Marine Cyanobacterium, *Lyngbya majuscula*

Thesis submitted by

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Declaration of Originality

I declare that the work presented in this thesis, to the best of my knowledge and belief is original and my own work, except as acknowledged in the text, and that the material has not been submitted, either in whole or in part for a degree at this or any other university.

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Abstract

Lyngbya majuscula is a filamentous marine cyanobacterium with a worldwide distribution in temperate and tropical regions to a depth of 30m. Over 70 chemicals have been isolated and characterised from this organism, many of which are biologically active. Dramatic responses have been elicited after human exposure to lyngbyatoxin A (LA) and debromoaplysiatoxin (DAT), toxins extracted from *L. majuscula*. These chemicals have been found to cause irritation at concentrations as low as 100 pmol and exposure of humans to this cyanobacterium in the environment is associated with irritant contact dermatitis, as well as eye and respiratory irritation. Previously, *L. majuscula* has been reported as implicated in negative health outcomes only in Hawaii and Okinawa. Recently large blooms of *L. majuscula* have occurred with increasing repetition in the Moreton Bay region as well as other areas along the eastern Australian coastline. A broad study of this organism and its potential effect on human health was undertaken.

LA and DAT and were found in samples of *L. majuscula* collected from Eastern Moreton Bay and North Deception Bay, Queensland, Australia, respectively. Samples of *L. majuscula* obtained from West Maui, Hawaii and the freshwater *L. wollei* from Florida contained LA. A quantitative measure of the irritant effects of the chemicals found in *L. majuscula* was made using a mouse ear-swelling test. The relative toxicities of two purified toxins, LA and DAT, were examined. These were found to produce swelling to a similar extent. The time course of inflammation and histopathological results were also similar for the two purified toxins. Less than 1 μg per ear of either toxin or a mixture (1:1) of the two toxins caused a measurable increase in ear thickness. When toxins were combined (1:1) there was an additive, not synergistic effect. Increases in ear thickness occurred

within 15 minutes. Crude extracts of *L. majuscula* from Moreton Bay were also applied to mice ears. The effect of crude extracts from Eastern Moreton Bay were not fully explained by the measured LA content, suggesting other toxin(s) and/or modulating factors were present. This effect was not found with *L. majuscula* containing DAT from North Deception Bay. Some samples of *L. majuscula* containing no measurable quantities of LA or DAT were found to exert an inflammatory response. This response had a different time course to response to LA or DAT.

In an effort to understand the potential exposure of humans to the toxins of *L. majuscula* the spatial and temporal distribution of LA and DAT were made in Eastern Moreton Bay and Northern Deception Bay. Not all samples of *L. majuscula* contained LA or DAT. More than ten-fold differences in DAT concentration were found at a single time at North Deception Bay (0.93-11.17 mg/kg freeze dry weight). LA was predominantly found at Eastern Moreton Bay while DAT was found only at North Deception Bay. Highest concentrations of toxin occurred when bloom size and density were also at their maximum (131.9 mg/kg LA and 43.0 mg/kg DAT). In an attempt to predict where *L. majuscula* biomass and toxins were maximal, and hence greatest chance of human exposure, a variety of physical and biotic parameters were obtained. Bloom intensity occurred when water temperature was maximal. Low precipitation periods were noted to occur before blooms. Total and dissolved reactive phosphorus were present at bloom initiation and peaks of chlorophyll-a in the water column were found at the peaks of *L. majuscula* bloom intensity.

In an attempt to understand the extent of human exposure to the toxic effects of *L. majuscula* several epidemiological studies were completed. To assess the potential affects a survey of the health of ocean users in the North Deception Bay

area, a residential area close to *L. majuscula* blooms, was undertaken. A postal survey was mailed to 5000 residents and a response rate of 27.4% was achieved. High numbers of people (78.2%) responding to the survey reported marine recreational water activity in Moreton Bay. Of those having marine recreational water activity, 34.6% reported at least one symptom, with skin itching the most reported symptom (22.7%) while fever was the least (0.4%). Younger participants had greater water exposure and symptoms than older participants. Participants with greater exposures were more likely to have skin and eye symptoms, suggesting agents in the marine environment contributing to symptoms. Of those entering Moreton Bay waters 29 (2.7%) reported severe skin symptoms, 12 of who attended health professionals. Six (0.6%) reported the classic symptoms of recreational water exposure to *L. majuscula*, severe skin symptoms in the inguinal region. Participants with knowledge of *L. majuscula* reported less skin, gastrointestinal and fever and headache symptoms.

Anecdotal evidence reported an outbreak of symptoms similar to those expected with exposure to *L. majuscula* on Fraser Island during the late 1990s. Examination of first aid records from Fraser Island revealed an outbreak of symptoms in January and February 1998. This coincided with the presence of a bloom of *L. majuscula.* The other four years examined had no *L. majuscula* blooms and the number of *L. majuscula* symptoms was much reduced.

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I would like to acknowledge several people without whose continued help and support this thesis would not be possible. Firstly, I would like to thank my principal supervisor Glen Shaw whose wealth of knowledge and enthusiasm on this topic are boundless. I would like to thank my associate supervisor Penny Webb who has patiently enlightened me in the study of epidemiology. I would like to acknowledge the support and helpfulness of all at the National Research Centre for Environmental Toxicology. I would like to acknowledge Judy O'Neil, Bill Dennison and Marine Botany, University of Queensland for their continued advice and comments, as well as resources and support. Geoff Eaglesham and Queensland Health contributed much to this thesis by assisting with measurement and characterisation of toxins. Healthy Waterways contributed many resources enabling completion of this thesis. Ian Marshall and Gerard Neville inspired me to investigate public health aspects of cyanobacteria more fully. The Bribie Island Environmental Society members assisted with the pilot surveys. The Queensland Parks Service contributed time and effort collecting samples and supplying information for this thesis. John Burns of the Cyanolab, Florida contributed by supplying samples and information on *Lyngbya wollei*. Professor R. E. Moore assisted by supplying standards to measure *Lyngbya* toxins. I would like to thank Ursula Keuper-Bennett for supplying samples of *Lyngbya majuscula* from Maui, Hawaii. Simon Forsythe of the School of Population Health assisted by contributing computer programs for data analysis. I would like to thank the Bureau of Meteorology for supplying weather data. Miles Yates and the Environmental Protection Agency contributed data on the presence of *Lyngbya* in Moreton Bay. Graham Webb of the Environmental Protection Agency and the Caboolture Shire Council contributed faecal coliform data. I would also like to thank the Environmental Protection Agency for use of their high volume air sampler.

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List of Abbreviations

ABS	Australian Bureau of Statistics
AT	aplysiatoxin
ATT	26(2'-aminoethylthio)-tetrahydroteleocidin A-2
BIEPA	Bribie Island Environmental Protection Association
bp	before present
DAT	debromoaplysiatoxin
Eims	electron ionisation mass spectrometry
EMB	Eastern Moreton Bay
C	carbon
GP	general practitioner
H & E	haematoxylin and eosin
IL	interleukin
kg	kilogram
LA	lyngbyatoxin A
LB	lyngbyatoxin B
LC	lyngbyatoxin C
LCMS	liquid chromatography/mass spectrometry
LD_{50}	lethal dose, 50% endpoint
LM	light microscopy
LPS	lipopolysaccharide
MS	mass spectrometry
MRWA	marine water recreational activity
µl	microlitres
µg	micrograms

N	nitrogen
Na	sodium
NDB	North Deception Bay
NHMRC	National Health and Medical Research Council
OR	odds ratio
P	phosphorus
ppb	parts per billion
ppm	parts per million
PSP	paralytic shellfish poison
PKC	protein kinase C
Si	silicon
SLS	sodium lauryl sulphate
TA	teleocidin A
TB	teleocidin B
TBTO	tri-N-butyl tin oxide
TEWL	transepidermal water loss
TN	total nitrogen
TNF	tumour necrosis factor
TP	total phosphorus
TPA	12-O-tetradecyanoylphrbol-13-acetate
TPTF	triphenyl tin fluoride

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1. Introduction

The thesis incorporates published information and original research on the marine cyanobacterium *Lyngbya majuscula* (Dillwyn) Harv. Gomont involving its toxicology, ecology and epidemiology. *L. majuscula* is present in South East Queensland and with increasing population and tourism in this area of Australia, interactions between these two entities could be well expected, and this is borne out by anecdotal evidence.

L. majuscula is a marine cyanobacterium with a worldwide distribution in tropical, subtropical and temperate waters. It forms long hair-like strands and grows to a depth of 30 m. It first came to the attention of medical scientists as the cause of dermatitis amongst recreational bathers in Hawaii during the 1950s. It has been a great source of natural products chemicals and many of these have been isolated and biological effects examined.

The thesis has been divided into several sections including:

Chapter one is an introduction to the thesis.

Chapter two introduces aims and objectives this thesis seeks to achieve.

Chapter three is a literature review which surveys published information on the toxicology, ecology and epidemiology of *L. majuscula*.

Chapter four describes original research carried out to examine the dermal toxicity of both purified dermatoxins from *L. majuscula* as well as from crude extracts from *L. majuscula* obtained from South East Queensland.

Chapter five describes original research examining environmental and ecological effects on the concentration of toxins of *L. majuscula* and cyanobacteria growth.

Chapter six describes examinations of pubic health effects of *L. majuscula.* Several experiments involving differing methodologies examine the health of populations having potential exposure to *L. majuscula.*

Chapter seven further evaluates the information from the preceding chapters and formulates conclusions drawn from them, including suggestions for future approaches.

2. Literature Review

2.1 Introduction

Marine cyanobacteria have a long evolutionary history (Hoffmann, 1999), with the oldest specimens found being 2.6-2.8 billion years old (Bartram *et al.*, 1999). Cyanobacteria, formerly termed "blue-green algae", are photosynthetic bacteria with widespread distributions in marine and freshwater environments (Bartram *et al.*, 1999). Interest in cyanobacteria has surged in recent years with the rising frequency and distribution of marine toxic cyanobacterial incidents (van Dolah, 2000). Reasons for this reported increase in harmful marine blooms have included: transport of toxic cyanobacterial cells and cysts in ballast water of ships; eutrophication (often associated with increased population); weather events such as drought, storm events and/or *El Niño*; and global climate change (van Dolah, 2000). Several genera of freshwater cyanobacteria pose risks to human health on multiple continents (Carmichael and Falconer, 1993; Ressom *et al.*, 1994; Hunter, 1998; Sivonen and Jones, 1999), however, sparse epidemiological data have made the assessment of risk to human health difficult (Philipp, 1991; Hunter, 1994; Pilotto *et al.*, 1997; Codd *et al.*, 1999; Pilotto *et al.*, 1999).

Cyanobacteria causes a variety of biological effects and, of the approximately 2000 species of cyanobacteria identified, 40 have been identified as toxic (Dow and Swoboda, 2000). Cyanobacteria have been found to cause toxicity in the hepato-, neuro-, gastro-intestinal and dermatic systems and have embryo-lethal, teratogenic, gonadotoxic (Kirpenko *et al.*, 1981), mutagenic (Collins *et al.*, 1981) and tumour promoting activities (Fujiki *et al.*, 1981). Toxins associated with the cyanobacteria are thought to have their evolutionary significance in protection against feeding by zooplankton, just as vascular plants have developed tannins, phenols, sterols and alkaloids to protect themselves from grazing. This hypothesis is supported by the fact

that zooplankton do not eat toxin-producing species if others are available and, when there are no others, they reduce intake of these toxic species (DeMott *et al.*, 1991; DeMott and Moxter, 1991). Grazing by protozoa on the unicellular marine alga (*Emiliania huxleyi*) has been shown to induce chemical defence (Wolfe *et al.*, 1997) and, in freshwater, a positive correlation has been shown between grazing pressure by *Daphnia* species and the toxicity of blooms dominated by the cyanobacterium, *Mircocystis* (Forsyth *et al.*, 1990). Alternatively, these "secondary metabolites", so called, as they do not appear to be physiologically essential, may have had some critical function in the past and their protective effect might in fact be incidental. This hypothesis is supported by their specificity in function or binding to internal cell machinery such as receptors or ion-channels (Carmichael, 1994; Mebs, 2001; Wright, 2002).

2.2 Toxic Cyanobacteria

Poisonings due to what appears to be cyanobacteria have been reported during the Han dynasty about 1000 years b.p. in Southern China (Bartram *et al.*, 1999). The first case of human disease due to cyanobacteria reported in the scientific literature was by Farre in London in 1844 where members of the genus *Oscillatoria* where found in faecal samples (Hunter, 1998). The first scientific report of animal fatalities due to a toxic algal bloom was recorded by Francis (1878) in Lake Alexandrina, South Australia, although documented reports extend as far back as 1853 (Codd *et al.*, 1994).

2.2.1 Hepatotoxins – Microcystins, Nodularins and Cylindrospermopsin

Microcystins are 7-member cyclic peptide hepatotoxins and have variable

chemical structures with substitution of L-amino acids, at 2 and 4, and demethylation of amino acids, at 3 and/or 7 (Moore, 1996). They are specific liver poisons in mammals, with acute doses causing liver haemorrhage and liver failure, and chronic doses are thought to promote growth of liver and other tumours (Bartram *et al.*, 1999). It has been suggested that these toxins may be the cause of high rates of liver cancer in China (Carmichael, 1994). Over sixty have been isolated and fully characterised from *Microcystis*, *Anabaena*, *Oscillatoria*, and *Nostoc* genera, as well as being found in terrestrial forms (*Hapalosiphon hibernicus*) (Prinsep *et al.*, 1992). Microcystins are absorbed across the ileum and uptaken via the bile transport system; they are taken up by hepatocytes and bind covalently to protein phosphatase 1 and 2A. This causes the microfilaments to collapse towards the nucleus. The collapse helps shrink the hepatocytes that normally touch each other and blood moves from the damaged capillaries, seeping into liver tissue. Symptoms of poisoning include weakness, anoxia, and pallor of membrane, vomiting, coldness and extreme diarrhoea. Death is by intrahepatic haemorrhage or hypovolaemic shock (Carmichael and Falconer, 1993). Pneumonia has been found to occur on inhalation of *Microcystis* toxins (Turner, 1990). Individual microcystins have been shown to have differential toxicities (Gupta *et al.* 2003).

Chronic administration of microcystin toxin to pigs increased mortality, liver disease (chronic exposure), and levels of alanine-a transferase in blood. Mortality showed a dose-response relationship, as did tumour incidence. Pigs on highest doses saw a decrease in weight gain, abnormal liver serum enzymes, as well as histological abnormalities (Falconer *et al.*, 1994).

Nodularins are the pentapeptide hepatotoxins produced by *Nodularia spumigena*, found in brackish waters in Australia (Francis, 1878), New Zealand

(Carmichael *et al.*, 1988) and the Baltic (Sivonen *et al.*, 1989). Toxicities vary from as high as LD_{50} 50μg/kg i.p. mice to non-detectable. Nodularin toxicity has a similar mechanism of action to microcystins (Sivonen and Jones, 1999).

The toxicity of *Cylindrospermopsis rackiborskii* was probably first recorded at Palm Island, North Queensland (Hawkins *et al.*, 1985). It has a molecular weight of 415 Da, and effects liver, kidney, thymus and heart. It is a potent inhibitor of protein synthesis and has been found to inhibit glutathione synthesis *in vitro*. Acute symptoms in animals included muscular fasiculatens, exaggerated abdominal breathing, cyanosis, convulsions and death within minutes, although maximum toxicity is usually observed after a period of 5 days (Terao *et al.*, 1994).

2.2.2 Neurotoxins

Anatoxin-a is a highly potent stereo-specific agonist of nicotinic-acetylcholine receptors with a LD_{50} of 10 μg/kg i.p. in mice (Kao, 1993). Muscles remain contracted in its presence, and stop working due to exhaustion by over stimulation. It has been found in a variety of genera including *Anabaena*, *Oscillatoria*, *Aphanizomenon*, *Cylindrospermopsis* and some strains of *Microcystis* (Carmichael, 1997). It has a homologue, homoanatoxin, of similar potency (Skulberg *et al.*, 1992).

Anatoxin-a(s), first found in *Aphanizomenon flos-aquae* blooms (Mahmood and Carmichael, 1986a) is a phosphate ester of cyclic N-hydroxygoamine with a LD_{50} of 20 μg/kg i.p. mouse. It induces salivation in mice, unseen in anatoxin-a symptoms (Mahmood and Carmichael, 1986b). The benthic freshwater species *Lyngbya wollei* produces saxitoxins and analogues. (Carmichael *et al.*, 1997; Onodera *et al.*, 1997). Australian varieties of *Anabaena circinalis* have been found to produce saxitoxins as well as other neurotoxins, C-toxins and gonyautoxins (Negri and Jones 1995; Beltran

and Neilan 2000).

2.2.3 Dermatoxins

A range of freshwater algae including *Anabaena*, *Aphanizomenon*, *Nodularia*, *Oscillatoria* and *Gleotrichia* can cause contact dermatitis of varying severity (Grauer and Arnold, 1961; Gorham and Carmichael, 1988; Soong *et al.*, 1992). Dermatitis caused by freshwater cyanobacteria is not allergenic in nature, but inflammatory. *L. majuscula* has been reported as the cause of dermatitis in swimmers for over forty years. Initial reports were during the summer months on the windward side of Oahu, Hawaii in an epidemic in 1958 (Banner, 1959; Chu, 1959; Grauer, 1959a; Grauer, 1959b; Grauer and Arnold, 1961). Substances from this species have also been implicated in severe oral and gastrointestinal inflammation (Sims and Zandee van Rilland, 1981).

2.2.4 Lipopolysaccharide Endotoxins

Cyanobacterial LPS differ from enterobacterial LPS by lacking a phosphate in the lipid A core and have been shown to be ten times less toxic (Hunter, 1998). LPS from gram-negative bacteria have been found to be pyrogenic and toxic (Kuiper-Goodman *et al.*, 1999). An outbreak of gastro-enteritis in Sewickley, Pennsylvania, in 1975 was reported in having its origin in cyanoabacterial LPS endotoxin (Keleti and Sykora, 1982). Lipopolysacchides from *Phormidium* and *Nostoc* may also have an inhibitory effect on certain inflammations (Garbacki *et al.*, 2000).

2.2.5 Allergic Reactions

Ressom *et al.* (1994) quotes Heise (1949) as being the first to confirm cases of

allergy-like symptoms with freshwater cyanobacteria of the *Oscillatoria* taxa following contact during swimming. Symptoms included hay fever, asthma, urticaria and conjunctivitis. Mittal *et al.*, (1979) found *Lyngbya major* provided positive skin test reactions in 25.7% of volunteers with naso-bronchial allergy (only 1.7% with *Oscillatoria simplicissma*). The species, isolated from air samples taken in Delhi included *Nostoc commune*, *Oscillatoria simplicissma*, *Anabaena fertilissima* and *Lyngbya major* (Mittal *et al.*, 1979). Further work needs to be undertaken in this area to identify differences between the allergic and irritant effects of exposure to members of Cyanophyta.

2.3 *Lyngbya majuscula*

While most toxic species of cyanobacteria are freshwater species, the marine *Lyngbya majuscula* (Gomont) has also been found to have highly toxic varieties (Mynderse *et al.*, 1977; Cardellina *et al.*, 1979). It has been found to contain over 70 biologically active components, many of which have been shown to be highly toxic (Table 2.1). *L. majuscula* is a benthic cyanobacterium that grows loosely attached to seagrass, sand and rocky outcrops. It grows in fine strands 10-30cm in length (Figure 2.1). It usually resembles a clump of drab, olive coloured hairy matted mass, composed of filamentous threads (Izumi and Moore, 1987; Dennison and Abal, 1999), although colour can vary from red to white to brown based on physiological state (Grauer and Arnold, 1961). *L. majuscula* occurs in abundance from the inter-tidal zone to a depth of 30m (Izumi and Moore, 1987). It has been found in estuarine and coastal waters in both tropical and subtropical regions (Table 3.2). *L. majuscula* was first reported in Queensland at Dunk Island in 1909 by E. J. Banfield, and in the Brisbane area in 1911 by S. T. White (Buchanan Heritage Services, 2003). *L. majuscula* and its toxicity have been

reported to have occurred for the majority of last century (Buchanan Heritage Services, 2003).

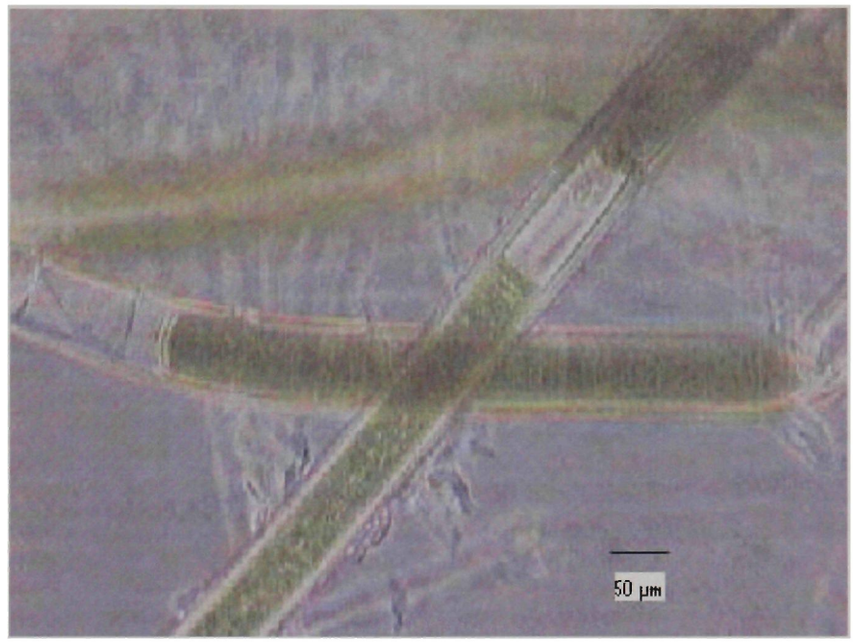


Figure 2.1 Photomicrograph of *Lyngbya majuscula* from North Deception Bay (phase contrast x 10).

Table 2.1 Chemicals extracted from *L. majuscula* (*isolated from *Gracilaria coronopifolia*, on which cyanobacteria are known to grow epiphytically, sea hares †*Stylocheilus longicauda* and ‡*Bursatella leachii*, which is known to feed on and accumulate toxins from *L. majuscula* and $^{\nabla}$cited as *Lyngbya* sp.).

Chemical	Author	Chemical	Author
acstamide 1-5	(Orsini *et al*., 2001)	lyngbyabellin A	(Luesch *et al*., 2000c)
antillatoxin	(Berman *et al*., 1999)	lyngbyabellin B	(Luesch *et al*., 2000b)
antillatoxin B	(Nogle *et al*., 2001)	lyngbyacarbonate	(Todd *et al*., 1995)
aplysiatoxin	(Fujiki *et al*., 1982)	lyngbyastatin 1	(Harrigan *et al*., 1998)
apramides A-G	(Luesch *et al*., 2000)	lyngbyatoxin A	(Cardellina *et al*., 1979)
apratoxin A	(Luesch *et al*., 2001)	lyngbyatoxin B-C	(Aimi *et al*., 1990)
barbamide	(Orjala *et al*., 1996)	majusculamide A-B	(Marner *et al*., 1977)
caylobolide A	(MacMillian and Molinski, 2002)	majusculamide C	(Carter *et al*., 1984)
carmabin A-B	(Hooper *et al*., 1998)	majusculamide D	(Moore *et al*., 1988)
cryptophycin	(Moore *et al*., 1996)	malyngamide A-C	(Cardellina *et al*., 1978)
curacin A	(Gerwick *et al*., 1994)	malyngamide D	(Gerwick *et al*., 1987)
curacin B-C	(Yoo and Gerwick, 1995)	malyngamide E-F	(Mynderse *et al*., 1978)
curacin D	(Marquez *et al*., 1998)	malyngamide G	(Praud *et al*., 1993)
debromoaplysiatoxin	(Mynderse *et al*., 1977)	malyngamide H	(Orjala *et al*., 1995)
debromogrenadiene	(Sitachitta *et al*., 1998)	malyngamide I	(Todd *et al*., 1995)
E-dehydroapratoxin A	(Luesch *et al*., 2000b)	malyngamide J-L	(Wu *et al*., 1997)
deoxymajusculamide D	(Moore amd Entzeroth, 1988)	malyngamide M-N*	(Kan *et al*., 1998)
dollastatin	(Luesch *et al*., 1999)	malyngamide O-P†	(Gallimore *et al*., 2000)
dollastatin 3	(Mitchell et al., 2000)	malyngamide Q-R	(Milligan *et al*., 2000a)
dragonamide	(Jimenez and Scheuer, 2001)	malyngamide S	(Appleton *et al*., 2002)
disydenamide	(Jimenez and Scheuer, 2001)	malyngamide T	(Nogle and Gerwick, 2003)
eicosanoid	(Moore, 1981)	malyngamide U-W	(McPhail and Gerwick, 2003)
gamma lactone	(Ainslie *et al*., 1986)	(-)-7-methoxydodec-4(*E*)-enoic acid	(Mesguiche *et al*., 1999)
grenadadiene	(Sitachitta *et al*., 1998)	microcolin A-B	(Koehn *et al*., 1992)
grenadamide	(Sitachitta *et al*., 1998)	monoterpene	(Todd *et al*., 1995)
hermitamide A-B	(Tan *et al*., 2000)	oscillatoxin A	(Mitchell *et al*., 2000)
indole derivative	(Todd and Gerwick, 1995)	pitiamide A	(Nagle *et al*., 1997)
indanone	(Nagle *et al*., 2000)	pitipeptolide A-B	(Luesch *et al*., 2001)
isomalyngbamide A-B	(Kan *et al*., 2000)	quinoline alkaloid 1-2	(Orjala *et al*., 1997)
kalkipyrone	(Graber and Gerwick, 1998)	somamides A-B	(Nogle *et al*., 2001)
kalkitoxin	(Berman *et al*., 1999)	tanoikolide	(Singh *et al*., 1999)
koroamide	(Mitchell *et al*., 2000)	ulongamide A-F$^{\nabla}$	(Luesch *et al*., 2002)
laxaphycin A-B	(Bonnard *et al*., 1997)	yacunamide A-B	(Sitachitta *et al*., 2000)
		ypaoamide	(Nagle *et al*., 1996)

Several versions of the taxonomic classification for *L. majuscula* have been attempted (Stanier and Cohen-Bazire, 1977; Rippka *et al.*, 1979), with the most recent being: Cyanophyceae, order: Oscillatoriales, family: Oscillatoriaceae, subfamily: Oscillatorioideae, genus: *Lyngbya*. *Lyngbya majuscula* has also been classified botanically as *Microcoleus lyngbyaceus* (Skulberg *et al.*, 1981). It has a variety of common names including "fireweed", "blanketweed" and "mermaid's hair". It has been described as differing from genus *Oscillatoria* by the obligatorily occurring

sheaths, which sometimes are lamellated. The filaments are very rarely falsely branched, waved, mainly wider than 6 μm, mainly compact, large leather strata (Anagnostidis and Komarek, 1988).

Table 2.2 Regions where *L. majuscula* has been recorded.

Region	Author	Location	Ocean/Sea
Australia	(Dennison *et al.*, 1999)	Moreton Bay	Pacific/Coral Sea
Curaçao	(Gerwick *et al.*, 1994)	CARMABI Beach	Caribbean
Fiji	(Williamson *et al.*, 2000)	Yacuna Island	Pacific
Florida	(Milligan, 2003b)	Dry Tortugas	Caribbean
France	(Mesguiche *et al.*, 1999)	Le Brusc	Mediterranean
Grenada	(Sitachitta and Gerwick, 1998)	Grand Anse Beach	Caribbean
Guam	(Nagle *et al.*, 1997)	Piti Bomb Holes	Pacific
Hawaii	(Banner, 1959)	Hawaii	Pacific
Madagascar	(Singh *et al.*, 1999)	Tanikeli Island	Indian
Marshall Islands	(Mynderse et al., 1977)	Enewetak Atoll	N. Pacific
Mozambique	(Silva and Pienaar, 1997)	Inhaca Island	Indian
Okinawa	(Hashimoto *et al.*, 1976)	Ryukyus Islands	Pacific
Palau	(Mitchell *et al.*, 2000)	Big Goby Marine Lake	Pacific
Palmyra Island	(Habekost *et al.*, 1955)		Pacific
Papua New Guinea	(Tan *et al.*, 2000)	Hermit Island	Pacific
Philippines	(Beutler *et al.*, 1990)	Batan Island	Pacific
Puerto Rico	(Ainslie *et al.*, 1986)	Mayaguez	Caribbean
Sri Lanka	(Grauer and Arnold, 1961)		Indian
Venezuela	(Koehn *et al.*, 1992)	La Blanquilla	Caribbean
Virgin Islands	(Marquez *et al.*, 1998)	St. Croix	Pacific

Genetic studies investigating the sequence homology between *Lyngbya* and other marine cyanobacteria species have been reviewed. Polyphasic taxonomy demonstrated a consensus between phenotypic, genotypic and phylogenetic taxonomy. Nelissen *et al.* (1994) analysed the sequence of 16S rRNA, which revealed that *Lyngbya* clustered with other cyanobacteria *Arthospira*, *Oscillatoria* and *Microcoleus*. Hoffmann and Demoulin (1991) recently described *Lyngbya bouillonii*, a reef-inhabiting cyanobacterium closely related to *L. majuscula*, found to occur in Papua New Guinea waters (Hoffmann and Demoulin, 1991). *L. bouillonii* also contains toxic compounds, as does the more distantly related freshwater species, *Lyngbya wollei* (Carmichael *et al.*, 1997). In contrast, the freshwater benthic *Lyngbya* isolates from the Murray-Darling Basin,

Australia, were found to be non-toxic (Baker and Humpage, 1994).

2.4 Toxicology of *L. majuscula*

Although first identified in 1912 in Hawaii (Banner, 1959), it was not until the late 1950s that *L. majuscula* was first implicated as the cause of an epidemic of a previously unreported acute contact dermatitis. During July and August 1958, 125 people were reported as suffering dermatitis on the windward beaches on northeast Oahu, Hawaii (Arnold, 1959; Chu, 1959; Grauer, 1959a, b) and this was attributed to exposure to *L. majuscula*. Dermatitis caused by *L. majuscula* was recorded in swimmers with symptoms similar to a burn, usually appearing in the genital, perineum and perianal areas. Initial symptoms of erythema and burning sensations, appearing a few hours after exposure gave way to blister formation and deep desquamation, lasting up to several days (Grauer and Arnold, 1961).

Histopathologic examination of human skin exposed to *L. majuscula* found acute, vesicular dermatitis consistent with contact dermatitis on topical application. Microscopic examination revealed superficial desquamation, oedema of the epidermis with vesicles of various sizes within the epidermis (stratum malpighii). Some vesicles contained polymorphonuclear leukocytes and red blood cells and the deepest portion of the epidermis was infiltrated by polymorphonuclear leukocytes. The superficial dermis showed infiltration of both chronic and acute inflammatory cells including mononuclear cells, eosinophils and neutrophils (Grauer and Arnold, 1961).

Grauer (1959a, b) showed that *L. majuscula* was the etiological agent that caused an acute toxic (esharotic) dermatitis by patch testing human volunteers. All 33 volunteers gave strong positive reactions to *L. majuscula* (Grauer and Arnold, 1961). These results were supported by Banner (1959) using similar patch testing and animal feeding and cutaneous trials that surmised that the dermatitis was toxic rather than

allergenic in nature. It reacted strongly in all patch test volunteers and it tested positive in the patch test of guinea pigs. Infants under 6 months reacted just as promptly and severely as people who had been swimming in the area and who had had previous eruptions. These results were later backed by the findings of Solomon and Stoughton (1978).

It was noted that dermatitis caused by *L. majuscula* followed a certain pattern of events. These included the affected individual swimming in water made turbid by suspended fragments of seaweed, followed by continued wearing of swimming suits after leaving the ocean and before showering. These events were followed by a gradual onset of itching and burning, which occurred within a few minutes to a few hours after emerging from the ocean. Visible dermatitis with redness began to occur after three to eight hours (Grauer and Arnold, 1961). The influence of this pattern of events preceding dermatitis caused by *L. majuscula* has since been questioned by the epidemiological data of Serdula *et al.* (1982).

In 1968, 242 of 274 (88.3%) people bathing at Gushikawa Beach, Okinawa, developed acute dermatitis. Their symptoms included itching, rash, burning, blisters and deep desquamation, causing pain. The areas most severely affected were the genitals, eyes, lips and adjacent areas (Hashimoto *et al.*, 1976). Although *L. majuscula* was known to occur at the beach, no samples were taken at the time. In September 1973, *L. majuscula* was sampled there and found to cause rashes and blistering on human skin (Hashimoto *et al.*, 1976). The toxic compound extracted was found to have almost identical properties to the uncharacterised toxin found by (Moikeha and Chu, 1971; Moikeha *et al.*, 1971), later shown to be a mixture of debromoaplysiatoxin (DAT) and aplysiatoxin (AT) (Fujiki *et al.*, 1985).

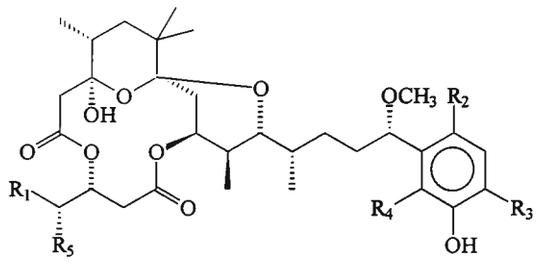
2.4.1 Toxicity of the *Lyngbya* toxin, debromoaplysiatoxin

The first report of the extraction of DAT from *Lyngbya* was isolated by chloroform extraction from *Lyngbya gracilis* (Mynderse *et al.*, 1977), from the Enewetak Atoll, the Marshall Islands, and characterised. This sample was later reclassified as *L. majuscula.* It had activity against P-388 mouse lymphatic leukaemia and was found to cause dermatitis. DAT and AT have been found to be active at similar concentrations (0.005 nmole per ear) (Fujiki *et al.*, 1983a).

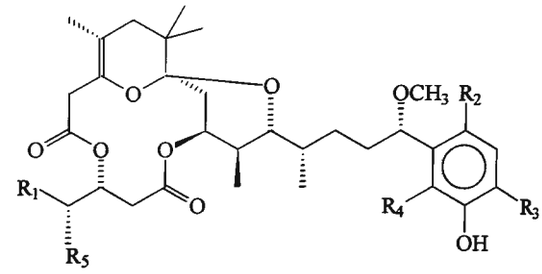
AT and DAT were first isolated from the digestive tract of the sea hare *Stylocheilus longicauda* (Kato and Scheuer, 1974; Kato and Scheuer, 1975). Accidental skin contact with sea hare toxin extract led to skin irritations. These phenolic bis-lactones have been shown to have potent tumour promoting activities, along with lyngbyatoxin A, another secondary metabolite of *L. majuscula.* All three substances produce erythema, blisters and necrosis. Aplysiatoxin and debromoaplysiatoxin are structurally identical except for the bromine on the aromatic ring (Figure 2.2). The anhydrotoxins of AT and DAT (no. 14-18, Figure 2.2) are relatively non-toxic (Moore *et al.*, 1984).

Examination of the structure-activity relationship of this hydrophobic region showed that the absence of the brominated molecule (DAT) in mixtures of *Lyngbya* toxins reduced malignant transformation and DNA synthesis in cells, although strength of binding to cell receptors was similar (Shimomura *et al.*, 1983). Although both DAT and AT appear to have the ability to cause dermatitis in a variety of animals and humans (Solomon and Stoughton, 1978), only AT has been shown to increase cell transformation and stimulate DNA synthesis *in vitro.* Ueyama *et al.* (1995) found both purified and commercial alpha$_1$-acid glycoprotein abolished *in vitro* activation of protein kinase C by DAT but not with AT.

Other species of the cyanobacteria Oscillatoraceae, including *Schizothrix calciola* and *Oscillatoria nigroviridis* contain DAT and other closely related compounds, including oscillatoxin A (31-nor DAT) (Moore, 1981). An aplysiatoxin-like substance, chemically related to the major toxin type in *L. majuscula*, has been found in marine *Schizothrix calciaola* (C. Agardh) Gomont (Entzeroth *et al.*, 1985). Nagai *et al.* (1997) showed that the structurally related compounds, the manauealides, were present in the red alga *Gracilaria coronopifolia* (Figure 2.1).



A



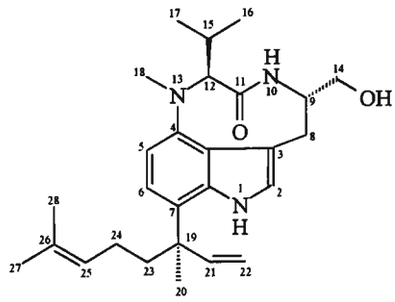
B

		Diagram	R1	R2	R3	R4	R5	mol. Weight
1	Aplysiatoxin	A	Me	Br	H	H	OH	671
2	Debromoaplysiatoxin	A	Me	H	H	H	OH	592
3	Oscillatoxin A	A	H	H	H	H	OH	576
4		A	H	Br	H	H	OH	655
5		A	H	Br	Br	H	OH	734
6		A	Me	Br	Br	H	OH	750
7	Synthetic I	A	Me	H	Br	H	OH	669
8	Synthetic II	A	Me	Br	Br	Br	OH	827
9	Synthetic III	A	Me	Br	H	Br	OH	748
10	Synthetic IV	A	Me	H	Br	Br	OH	748
11	Manauealide A*	A	Me	H	Cl	H	OH	626
12	Manauealide B*	A	Me	H	Br	H	OH	670
13	Manauealide C*	A	Me	H	H	H	COCH3	634
14	Anhydro AT	B	Me	Br	H	H	OH	654
15	Anhydro DAT	B	Me	H	H	H	OH	575
16	Anhydro oscillatoxin A	B	H	H	H	H	OH	558
17	Anhydro	B	Me	Br	Br	H	OH	732
18	Anhydro	B	Me	Br	Br	Br	OH	813

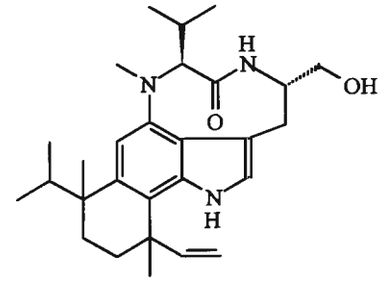
Figure 2.2 Debromoaplysiatoxin and its analogues (*extracted from *Gracilaria coronopifolia)*

2.4.2 Toxicity of the *Lyngbya* toxins, Lyngbyatoxin A, B and C

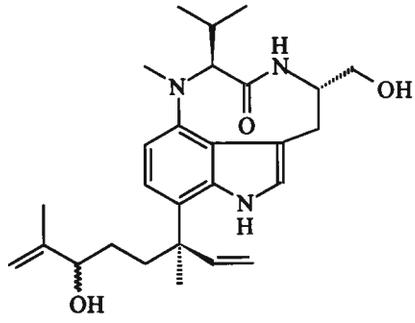
The structure of LA (Figure 2.2) was initially determined from a shallow-water variety of *L. majuscula* (Gomont) collected at Kahala Beach, Oahu, Hawaii (Cardellina *et al.*, 1979). The toxin content was 0.02% of freeze-dried weight. The structure of LA is identical to an isomer of teleocidin A (TA) found in the mycelia of several strains of *Streptomyces* (Takashima *et al.*, 1962). TA was discovered when workers in an industrial process that produced antibiotics complained of a skin irritation and the antibiotic was found to be infected with *S medicocidcus* (Sugimura, 1986). Investigation of the biological effect of LA found it had a minimum lethal dose (LD_{100}) of 0.3mg/kg by the intra peritoneal (IP) route in mice, comparable with teleocidin B (LD_{50} = 0.22mg/kg IP in mice) (Figure 2.2), another toxin from *Streptomyces*. Epimers of teleocidin based around C19 have been shown to exist in *S. medicocidcus* but only the 19*R* epimer was found in *L. majuscula* (Aimi et al., 1990). Inactivation occurred on hydrolysis of LA and TB (Moore, 1981; Moore *et al.*, 1984). Dermonecrotic and tumour promoting activity was found only to be obtained if the stereochemistry at C-9 and C-12 in both LA and TB was S,S (Figure 2.2). Fujiki *et al.* (1983a) found LA caused a reddening in the ears in 50% of the mice tested via topical application at the concentration of 0.011 nmole per ear.



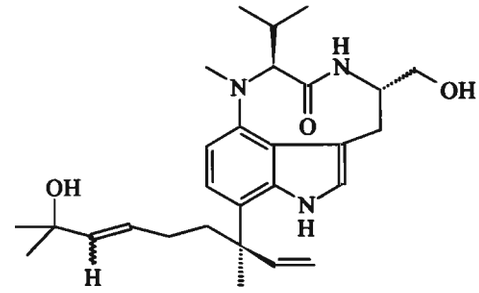
LA



TB



LB



LC

Figure 2.3 Structures of lyngbyatoxin A, teleocidin B, lyngbyatoxin B and lyngbyatoxin C.

Stafford *et al.* (1992) examined whether conclusions could be drawn from the skin penetration of LA and the n-octonol/water partition coefficient of this toxin. LA is slightly lipophilic (mean log n-octonol/water partition coefficient 1.53) and penetration of LA as a percentage of dose was found to be 23 and 6.2 percent for guinea pig and human skin respectively, over one hour (Stafford *et al.*, 1992).

Lyngbyatoxin B (LB) and lyngbyatoxin C (LC) were isolated from *L. majuscula* collected at Kahara Beach, Oahu, Hawaii (Aimi *et al.*, 1990). LB and LC

were found to have different activities from LA. The 50% inhibition for specific binding of ^{3}H-12-*O*-tetradecanoylphorbol-13-acetate (TPA), another tumour promoter and antagonist of protein kinase C (PKC) receptor, occurred at ED_{50} 2.2 μM and 0.2μM for LB and LC, respectively, corresponding to 1/200th and 1/20th the activity of LA (Aimi *et al.*, 1990).

Kozikowski *et al.* (1991) explored the use of synthetically produced structural analogues of LA to ascertain information on the structure-activity relationships of this group of toxins. Introduction of an –OH group on the C-7 decreased the ability of the molecule to activate PKC in comparison to TPA (256±48 to 899±104 PKC activity pmol min^{-1} (mg of protein)$^{-1}$, respectively). The addition of the OH group was interpreted as a decreased ability to associate with the membrane bilayer.

2.5 Factors Effecting Toxicity

Growth of cyanobacteria is affected by its environment and hence the numbers of organisms capable of producing toxin. The environment also affects the concentration per individual cell and the ability of cells to release toxin (Sivonen, 1996). A major environmental parameter effecting growth is light (Sivonen, 1990; Rapala *et al.*, 1993b). Light demand differs between the taxa of cyanobacteria, with those of *Oscillatoria* generally low, *Anabaena* medium and *Aphanizomenon* high.

Amount of light is linked to water temperature with toxin production in cyanobacteria was found to be high at 18-25 C, but depressed below 10 C, and above 30 C (Sivonen, 1996 Jones and Sivonen, 1999). Robarts and Zohary (1987) examined literature concerning the effect of temperature on the cyanobacteria *Anabaena*, *Aphanizomenon*, *Microcystis* and *Oscillatoria*. All were found to be temperature dependent in respect to photosynthetic capacity, specific respiration and growth rates,

with optima being at 25 C and above. The four genera varied in response to low temperatures. Temperature effects were found to be secondary to indirect temperature effects such as degree of water mixing and nutrients in determining the dominance of bloom forming cyanobacteria. Production of different microcystins has been noted at different light intensities and temperatures (van der Westhuizen *et al.*, 1986; Rapala *et al.*, 1995).

It has been reported that in shallow turbid lakes and reservoirs with high nutrient concentrations and high total nitrogen:total phosphorus (TN:TP) ratios, the dominant cyanobacteria are often taxa such as *Oscillatoria* and *Lyngbya* (Cichra *et al.*, 1995; Havens *et al.*, 1998). They aimed to show that underwater irradiance determines dominance of bloom forming (high light adapted) or non-bloom forming (low light adapted) taxa. The model proposed by Havens *et al.* (1998) for subtropical lakes mirrors that of Reynolds (1984) for English lakes. Havens *et al.* (1998) found in shallow eutrophic lakes, the dominant species varied alternatively from low light adapted taxa such as (*Oscillatoria* and *Lyngbya*) to high light adapted taxa such as *Anabaena*, *Microcystis* and *Aphanizomenon.*

Yoshida *et al.*, (1995) found *Lyngbya contorta* dominant in inland freshwaters when water temperatures are high, total N: total P is high, ratio of total N:dissolved inorganic nitrogen is high and total P:dissolved inorganic P ratio is high. Equally important was low inorganic dissolved N to dissolved inorganic P and low total N to total P ratios. Hepatotoxin production increases at high P concentrations. P has been linked to the limiting factor in on growth of cyanobacteria. No effect was seen with anatoxin-a. Similarly *Microcystis* and *Oscillatoria* produce greater quantities of microcystins when levels of N are high (Sivonen, 1990). Other factors shown to affect growth include pH of growth environment (van der Westhuizen and Eloff,

1983) and iron limitation (Lukac and Aegerter, 1993).

Carmichael (1986) suggested that cyanobacteria may produce toxins in response to stresses such as nutrient limitation or grazing. Similar trends have been observed with secondary metabolites in vascular plants (Waterman and Mole, 1994). Sadek *et al.* (1986) reported that a bloom of *Lyngbya limnetica* (775 000 organisms/L) caused mass mortality of fish kept in ponds for aquaculture at an Egyptian oasis. Effluent from an iron crusher factory was used in the ponds and contained 0.07±0.25 mg Fe/L at pH 7.8±0.6. It has been noted that in the species *Lyngbya majuscula*, samples giving a positive toxicity reaction have been red in colour, compared to the usual olive drab colour. This change in colour is well known in cyanobacteria, with the normal pigment phycoyanin being reduced to the red pigment phycoerythrin, usually under conditions of reduced oxygen tension (Banner, 1959).

Anatoxin-a and hepatotoxins are retained within the cyanobacterial cells when conditions for growth are favourable. The concentrations increase during the logarithmic growth phase and reached maximal levels in the late logarithmic phase (Sivonen, 1996). Orr and Jones (1998) and Long *et al.* (2001) suggest that microcystin production is controlled by the environmental effects on the rate of cell division, not through a direct effect on the metabolic pathway. This group also showed the amount of microcystin contained in a single cell of *Microcystis aeruginosa* varies within a two- to three-fold range. Many cyanotoxins are only released once cells begin decomposing. This can occur on the natural breakdown of the cyanobacterial bloom or on the artificial lysis of a bloom by application of $CuSO_4$ (Lam *et al.*, 1995).

2.6 Human Health Effects Associated with Exposure to *L. majuscula*

After an outbreak of "seaweed itch" or dermatitis escharotica on the windward side of Oahu in the last two weeks of August 1980, Hawaii Department of Health conducted a descriptive epidemiological study (Serdula *et al.*, 1982). Hospital emergency rooms and physicians in the Kailua area were notified and requested to report cases with symptoms of skin or eye irritation within 24 hours after bathing at a windward beach to the authors. A questionnaire was administered to cases to gather information on symptoms, exposure times and other factors that may have lead to increased symptoms.

Rashes and blistering occurred in 31 of 35 questionnaire respondents and were similar to those described by other authors (Grauer and Arnold, 1961; Solomon and Stoughton, 1978). They were noticed 4-20 hours after water exposure and persisted for 2 to 12 days. In 45%, symptoms were sufficiently severe that they attended a physician. Symptoms occurred in bathers who had been in the water from 2 minutes to 4 hours and there was no association between severity of symptoms and the length of time in the water. Symptoms occurred on exposed areas of the body as well as areas covered by a swimsuit leading the authors to question whether exposure to the toxin occurs through contact with toxic lipophilic exudates released by the cyanobacterium, rather than with the cyanobacterium itself. Rinsing in freshwater, which had previously been noted to reduce the occurrence of dermatitis (Grauer and Arnold, 1961), was ineffective in two cases. It was concluded that the only guaranteed protection against seaweed itch was to stay out of the water.

Of particular interest were members of a picnic party who had similar exposures. Of those attending, 20 entered the water and 15 of those who entered the water had symptoms. No significant difference between those affected or unaffected was found with respect to the time they had spent in the ocean, the interval between leaving the

water and showering, and the interval between leaving the water and removal of the swimming suit. DAT and AT were extracted from *L. majuscula* samples collected at the beach. It was not reported if examinations were made to identify other toxins.

A second study was conducted in 1983 during an outbreak of an airborne eye and respiratory irritation in Lahaina, Maui, Hawaii (Anderson *et al.*, 1988). Health surveys were administered to employees of commercial establishments along the main street of the area affected by the outbreak, one week after the highest reporting occurred. In addition, physicians in the Lahaina region were also interviewed with respect to recent cases of contact dermatitis ("swimmers itch") and unexplained eye and respiratory irritation they had seen.

This study was able to eliminate other potential sources of eye and respiratory irritation such as car emissions, discharge of toxic chemicals from local industry, pesticide application, cane burning and discharge of thermal cooling water as the cause of the outbreak. Air monitoring showed levels of carbon monoxide, ozone and nitrogen dioxide below US Federal levels and no irritating chemicals were found in storm drains. Inspection of the waterfront revealed no evidence of fish kills, nor the presence of other potentially irritating organisms. Other cyanobacterial blooms that have become airborne have been reported to cause respiratory irritation (Hawser *et al.*, 1991). Reports of 19 cases of "seaweed dermatitis" indicated that *L. majuscula* was present in the area and that the variety was toxic. High volume air filter sampling revealed fragments of *L. majuscula* present in the air and scrapings of windows in the waterfront area revealed *L. majuscula* strands. One patient died of interstitial pulmonary oedema during this time, and it has been speculated that his was possibly related to inhalation of fragments of *L. majuscula* (Izumi and Moore, 1987). The authors stressed that the most important factor in this outbreak was probably the

unusual meteorological conditions. The presence of tropical storm Gil led to high surf in the Lahaina region, and this, combined with high tides, led to the formation of sea spray mists (Anderson *et al.*, 1988).

Other incidents of exposure to aerosolised *L. majuscula* include walkers on Gushikawa Beach, Okinawa, who developed facial rash, conjunctivitis, inflamed eyes and lacrimation. *L. majuscula* was present in the area and retrospectively suggested as the cause (Hashimoto, 1979). There have also been reports of facial rash and eye irritation after driving on a beach covered with *L. majuscula* on Fraser Island, Australia (Dennison, personal communication). Eye and respiratory irritation have also occurred after cleaning dried *L. majuscula* from fishing nets and crab pots after fishing in Moreton Bay, Australia (Dennison and Abal, 1999) and fishers in Hawaii have long recognised the relationship between *L. majuscula* in their nets and the onset of skin irritation (Grauer and Arnold, 1961) (Table 2.3).

Vibrio species, *V. alginolyticus* and *V. parahemolyticus* have been cultured from marine specimens of *L. majuscula*, as well as specimens recovered from the beach (Sims *et al.*, 1993). These bacteria have been found in the past to produce blisters in a number of patients. Cholera outbreaks, caused by another species of this genus, *V. cholerae*, have been long associated with coastal cyanobacterial blooms (Epstein, 1993).

Table 2.3 Documented occurrences skin, eye and respiratory irritation by *L. majuscula*.

Date	Location	Country	No. cases	Author
1958	Laie	Hawaii	123	(Grauer and Arnold, 1961)
1958	Kaaawa	Hawaii		(Grauer and Arnold, 1961)
1958	Kailua Bay	Hawaii		(Grauer and Arnold, 1961)
1958	Waimanalo Beach	Hawaii		(Grauer and Arnold, 1961)
1959	Laie	Hawaii		(Grauer and Arnold, 1961)
1960	Laie	Hawaii		(Grauer and Arnold, 1961)
1968	Okinawa	Japan	242	(Hashimoto *et al.*, 1976)
1973	Okinawa	Japan		(Hashimoto *et al.*, 1976)
1976	Laie Bay, Oahu	Hawaii		(Mynderse *et al.*, 1977)
1980	Kailua, Oahu	Hawaii	86	(Serdula *et al.*, 1982)
1980	Kalama, Oahu	Hawaii		(Serdula *et al.*, 1982)
1980	Pilapu, Oahu	Hawaii		(Serdula *et al.*, 1982)
1983	Maui	Hawaii	31	(Anderson *et al.*, 1988)
1986	Oahu	Hawaii		(Izumi and Moore, 1987)
1996-7	Moreton Bay	Australia		(Dennison *et al.*, 1999)
1997-8	Moreton Bay	Australia		(Dennison *et al.*, 1999)

2.7 Human Toxicity from Consumption of *L. majuscula*

Accidental human oral ingestion of *L. majuscula* while consuming seaweed led to an instant burning sensation and several hours later the mucous membrane in the anterior portion of the mouth appeared to be scalded (Sims and Zandee van Rilland, 1981). Discomfort lasted for three days and had usually completely disappeared in three weeks (Marshall and Vogt, 1998). In Hawaii, accidental ingestion of *L. majuscula* by humans may occur, as it resembles the edible alga *Enteromorpha prolifera* (Limu 'ele' ele) and also grows epiphytically on some edible seaweed, with its filaments attaching to seaweed thalli (Moore, 1981). Nagai *et al.* (1996) reported that DAT and AT were also present in samples of the red alga *Gracilaria coronopifolia* J. Agardh (Gracilariaceae) causing symptoms in humans including burning sensation in the mouth and throat, vomiting and diarrhoea. The true origin of these toxins was, however, later attributed to *L. majuscula* (Ito and Nagai,

1998).

Meat from the turtle *Chelonia mydas* in Madagascar has been implicated in the fatal intoxication of a man (Yasumoto, 1998). The toxin LA was later identified in the meat by liquid chromatography/mass spectrometry.

Fish, including surgeonfish, butterflyfish, damselfish and pufferfish, from the Palmyra Island, 960 nautical miles SW of Honolulu, have been found to be toxic (Dawson *et al.*, 1955). *L. majuscula* in the area was found to be moderately toxic using intraperitoneal injections of aqueous extracts in mice. It was found to be part of the intestinal contents of a variety of species of fish including 38 of 71 poisonous fishes examined. Parts of these fish were rated between non-toxic to strongly toxic. Toxicity occurred in extracts of muscle, liver, gonads, intestines, viscera, and skin (Dawson *et al.*, 1955; Habekost *et al.*, 1955).

Hawaiian fishermen from Molokai and Kauai have suggested the toxicity of the Hawaiian mullet was due to the fish eating *L. majuscula* (Helfrich and Banner, 1960). Intoxication followed consumption of the flesh of this fish, especially the head of the mullet and surmullet ("nightmare weke") and caused nightmares and hallucinations.

Rabbitfish, *Siganus fuscescens* have been noted to feed on seagrass entangled with *L. majuscula*, where it growths epiphytically (Hashimoto *et al.*, 1976) and the authors noted that rabbitfish had been found to become toxic when they inhabited certain coral reefs. The symptoms were different from ciguatera poisoning (Hashimoto *et al.*, 1976).

Hawaii has the highest incidence of gastrointestinal cancer in the world (Moore, 1984). At least two seaweeds in the Hawaiian diet contain halogenated carcinogenic and mutagenic compounds (*Asparagopis taxiformis* (limu kohu) and

Laurencia nidifica (limu mane'ono'o))(Moore, 1984). Frequent ingestion of such compounds could increase probability of gastrointestinal cancer, especially if combined with tumour promoters found in *L. majuscula*. Although the toxins of *L. majuscula* are potent tumour promoters, neither skin tumours, nor gastrointestinal cancers have been reported in humans exposed to LA and DAT from *L. majuscula* (Fujiki *et al.*, 1981).

2.8 Toxicity of *L. majuscula* in Other Organisms

Lightner (1978) found *L. majuscula* caused mass mortality of raceway-reared blue-shrimp *Penaeus stylirostris*. Pathology was characterised by necrosis of the lining of epithelium of the midgut, dorsal caecum and hindgut as well as haemolytic enteritis.

Experiments by (Sundararaman *et al.*, 1994) showed a cultured marine *Lyngbya* species contained one or more toxins that deter food intake, and causes decrease in body weight, when the cyanobacterium was fed to rats at 5mg/day. Haematological characteristics were examined and positive bioactivity without haematological changes was interpreted as a pharmaceutical effect of toxins.

AT applied by oral or intra peritoneal routes in mice at low dose caused diarrhoea in the large intestine, where the submucosa accumulated fluid. This fluid moved into the lamina propria and then into the lumen as the surface epithelial cells were broken. The caecum was then the main target. After the diarrhoea ended, there was a large increase in the number of Goblet cells and many cracks were left on the surface of the epithelium. The target site of a lethal dose was the whole small intestine, where the toxin caused bleeding resulting in haemorrhagic shock (Ito and Nagai, 1998).

2.9 Differential Toxicology of *Lyngbya majuscula*

It is currently unknown why some strains of *L. majuscula* are toxic and others are not, as few gross differences between the strains have been noted (Izumi and Moore, 1987). Banner (1959) reported that no differences between toxic and non-toxic varieties were considered important enough to be used for a systematic separation into different species.

As early as 1959, it had been noted that although *L. majuscula* was found on several islands of the Hawaiian chain, toxic strains were only found in a few localities (Banner, 1959; Chu, 1959). Differences in toxicity were noted using 6-hour patch tests on human skin with alcoholic extracts of *L. majuscula* collected from swimming beaches about the island of Oahu, Hawaii (Banner *et al.*, 1960).

Strains from the windward side of Oahu were found to contain DAT and AT while those from Kahala Bay on the leeward side of Oahu produced primarily LA (Cardellina *et al.*, 1979). *L. majuscula* on the leeward side of has never been implicated with an outbreak of seaweed dermatitis (Izumi and Moore, 1987). Moore (1984) identified the presence of LA in a variety of *L. majuscula* growing at Kahala Beach, Diamond Head, Oahu, Hawaii even though swimmers in the Kahala area had reported no seaweed dermatitis. This could be due to other factors such as the winds blowing the cyanobacterium away from the beach, the type of water activity, or bathers not reporting health outcomes. Interestingly, cyanobacterial samples collected from the seaward side (with presumably stronger weather conditions) of Enewetak were much more toxic than a specimen collected on the lagoon side of the island (Mynderse *et al.*, 1977). AT was not a constituent of the *Lyngbya* from Enewtak Atoll (Mynderse *et al.*, 1977).

Toxic varieties have been twice discovered to be red-coloured instead of the

usual drab olive colour. Under conditions of decreased oxygen tension the normal pigment phycocyanin may be reduced to the red pigment phycoerythrin (Banner, 1959). This differs from *L. wollei* where the healthy, greenish blue-black coloured filaments were toxic while those that had been abraded into heterogeneous ball-like material of a brownish yellow colour were nontoxic. Both benthic and surface mats *L. wollei* have been found to contain saxitoxins (Carmichael *et al.*, 1997).

2.10 Ecological Implications of *L. majuscula* in Marine and Terrestrial Environments

The presence of cyanobacterial blooms containing high quantities of toxic chemicals has important implications for both human and animal populations. Many chemicals found in *L. majuscula* have been shown to act as feeding deterrents in a number of marine species (Nagle *et al.*, 1996; Pennings *et al.*, 1996; Thacker *et al.*, 1997; Nagle *et al.*, 1998; Nagle and Paul, 1998, 1999) and non-marine species (Banner, 1959; Sundararaman *et al.*, 1994). Non-marine species can come into contact with *L. majuscula* when it becomes washed up in wracks on the shoreline. This has probably been best exemplified in the case where *Lyngbya* caused the death of horses feeding on it on beaches in Sri Lanka (Grauer and Arnold, 1961).

Cyanobacteria containing tumour promoters have been implicated in the debilitating, neoplastic disease of marine turtles, fibropapillomatosis (Landsberg *et al.*, 1999). Immature green turtles feed primarily on algae of the *Gracilaria* species (Brand-Gardner *et al.*, 1999), on which *L. majuscula* is known to grow epiphytically (Nagai *et al.*, 1996). The increase in incidence of fibropapillomatosis in green turtles (*Chelonia mydas*) in Moreton Bay, Australia, (Aguirre *et al.*, 1999) may be linked to the increase in the number and/or size of large *L. majuscula* blooms occurring in this

area. Fibropapillomatosis is also common among this species of turtle in regions of Hawaii and Florida, which are also prone to blooms of *L. majuscula*. The aetiology of fibriopapillomatosis is unknown but has been linked to oncogenic viruses (Quackenbush *et al.*, 1998). Tumour promoters, such as those found in *L. majuscula*, have been shown to enhance virus synthesis (Wunderlich *et al.*, 1984), enhance onocogene-induced transformation of cells (Hsiao *et al.*, 1984; Hsiao *et al.*, 1986), as well as reduce immune responses by suppression of the immune-surveillance mechanism (Yamashita, 1985). Other chemicals in *L. majuscula* also have immunosuppressive properties (Koehn *et al.*, 1992; Zhang *et al.*, 1997).

Harmful marine algal blooms have been implicated wholly or in part for the mass deaths of marine animals in the past (Hernandez *et al.*, 1998; Scholin *et al.*, 2000). This increase in marine diseases has purported to be linked to increased physiological stresses compromising host resistance and increased frequency of opportunistic diseases due to changes in climate and anthropogenic factors (Harvell *et al.*, 1999). Only with increased knowledge cyanobacterial blooms and their toxicologies will the future effects on humans and ecological health be able to be predicted.

3. Dermal Toxicology of *Lyngbya majuscula*

3.1 Introduction - Toxicology

Cyanobacteria have been shown to be a rich source of biologically-active compounds, many of which are peptides or hybrids of polypeptide biosynthesis (Moore, 1996). *L. majuscula* has also been found to contain a wealth of bioactive chemical species and has become a favourite of natural product chemists searching organisms for potential pharmaceuticals (Faulkner, 1999; 2000) (Table 2.1). The variety of biologically active chemicals also increases the potential for toxicological properties of individual chemicals as well as the interaction between chemicals and biological mechanisms.

L. majuscula was first identified as toxic in the middle of the last century (Dawson *et al.*, 1955; Habekost *et al.*, 1955). Although identified in the 1912 in Hawaii, *L. majuscula* was not implicated as causing dermatitis until the late 1950s after an epidemic of acute contact dermatitis (Grauer, 1959a; Grauer and Arnold, 1961). All 33 human volunteers patch tested with *L. majuscula* gave strongly positive vesicular reactions. Experiments by Banner (1959) and Grauer and Arnold (1961) suggested that the nature of dermatitis was contact rather than allergenic in nature.

This allowed the supposition that a toxic agent present in *L. majuscula* was the aetiology of the episodes of dermatitis. Chu (1959) found that the toxic substances associated with this species were stable in cultured cyanobacteria for up to two months. Moikeha and Chu (1971) and Moikeha *et al.* (1971) isolated toxic factors from *L. majuscula* that demonstrated dermo-necrotic activity following injection into the abdominal skin of mice and guinea pigs. Mynderse *et al.* (1977) and Cardellina *et al.* (1979) identified and characterised debromoaplysiatoxin (DAT) and lyngbyatoxin A (LA), respectively. LA was found to have a structure identical to the toxic dermal

irritant teleocidin, previously extracted from *Streptomyces* (Takashima *et al.*, 1962). The aplysiatoxins had previously been found in the marine mollusc *Stylocheilus longicauda* (Kato and Scheuer, 1974). Soon after LA (Nakayasu *et al.*, 1981) and DAT (Fujiki *et al.*, 1983a) were found to be tumor promoters.

Solomon and Stoughton (1978) performed the initial toxicological studies with purified DAT and found it did indeed produce an irritant pustular folliculitis in humans and severe cutaneous inflammatory reaction in rabbits and mice. Only very small quantities of purified toxin were needed to induce inflammatory reactions in humans (500 ppm or 0.5 μg/ml in ethanol) and rabbits and mice (0.5 ppm). This group also examined the histopathology of DAT.

LA (1 μM) was found to cause contraction of vascular smooth muscle (rabbit aorta) by an extracellular and intracellular calcium-. endothelium and neuron-independent mechanism similar to other protein kinase activating phorbol esters (Robinson *et al.*, 1991).

More recently the oral toxicology of *L. majuscula* and purified toxin derived from this organism have been examined in mice (Ito and Nagai, 1998; Ito and Nagai, 2000; Ito *et al.*, 2002). LA had an i.p. lethal dose of 250 μg/kg in immature mice while no deaths occurred in mature mice at 300 μg/kg. No lethality occurred at p.o. 1000 μg/kg. At p.o. 600 μg/kg enhanced mucous secretion and light erosion occurred after ten minutes in the stomach and small intestine. Effective dose levels were found to be similar between LA and AT (Ito *et al.*, 2002).

The proposed mechanism of action of the toxins LA and DAT is via protein kinase C mediated receptors. Amongst other properties these non-phorbol ester tumour promoters cause a reversible decrease in transepithelial voltage and transepithelial resistance at concentrations as low as 10^{-8} M. Transepithelial

permeability is controlled by regulation of tight junctions which in turn are mediated by protein kinase C (Mullin *et al.*, 1990).

Due to the radically different chemical structures of LA and DAT it was proposed that they may also work via other receptors/mechanisms. Differential activities of LA and DAT have been noted by several authors (Fujiki *et al.*, 1981; Fujiki *et al.*, 1983b; Fujiki *et al.*, 1990), although some have noted similarities (Fujiki *et al.*, 1981; Moore *et al.*, 1986).

3.2 Aim

The aim of this study was to establish an animal model for dermal toxicity that would elucidate the toxicity of Queensland *L. majuscula* strain. It was proposed that purified LA and DAT samples obtained from Prof. Richard Moore, University of Hawaii, would be used to establish a dose-response curve against which toxicology of Queensland *L. majuscula* could then be determined. The potency of LA and DAT in regard to dermal toxicity could then be established as well elucidating if the entire toxicity of *L. majuscula* from Queensland was due these two toxins. It was proposed to examine any synergistic effect by LA and DAT on toxicity. A comparison of differences in histopathology of LA and DAT was proposed in order to compare other states of dermal toxicity.

3.3 Methods - Toxicology

3.3.1 General

LA and DAT standards were obtained from Prof. Richard Moore, University of Hawaii. Crude extracts of *L. majuscula* were isolated from *L. majuscula* collected from Moreton Bay, Queensland. Cyanobacteria were stored in seawater (4-8 hours) until returned to the laboratory and frozen and stored (0°C). On thawing, cyanobacteria were rinsed with distilled water and refrozen for lyophilisation. Frozen samples of *L. majuscula* (~20g) were lyophised until dry and ground using a coffee grinder (Phillips, Australia). Samples were extracted with 50 ml acetone (EM Science, Gibbstown, USA) overnight, with 20 minutes of sonication during this time. Extract was then filtered through glass fibre filter paper (Whatman, Maidstone, UK) under vacuum into a Büchner flask to remove particulate matter. Solvent was then removed by rotary evaporation (Büchi, Switzerland) with temperature maintained at less than 40°C. Extracts were solubilised in 50% methanol and passed through a 0.45um Millex-HA (Millipore, Milford, USA) syringe driven filter unit before testing for the presence of LA and DAT.

Appropriate quantities of toxin in aqueous methanol were selected to give desired concentrations in 20 µl and dried under a stream of nitrogen. Dimethyl sulphoxide (DMSO) (AMRESCO, Solon, USA) was used to dissolve extracts and purified *L. majuscula* toxin at appropriate concentrations and apply to mice ears. Solutions were prepared immediately prior to application to mice ears. All chemicals used were of analytical standard or higher.

3.3.2 Mice

Six to eight week female specific pathogen free (SPF) BALB/c mice were obtained from University of Queensland, Australian National University, Curtain University and University of Adelaide animal breeding programmes. Mice were maintained under Queensland Health Ethics Committee guidelines. All mice were housed in plastic boxes with wood shavings at 21±3°C and 55±10% humidity with a 12-hour light/dark cycle at animal facilities at Queensland Health Scientific Services and National Research Centre for Environmental Toxicology. Standard pelletised diet (Narco Pty, Rocklea, Brisbane, Australia) and tap water were supplied *ad libitum*. Mice weighed 15.8-23.1 g at the start of the study and were weighed daily during the trial period. Tail marks were used for identification.

3.3.3 Mouse Ear Swelling Test (MEST)

Although MEST was originally developed as a murine model of the elicitation phase of a hypersensitivity response, it is used here referring to the test of irritancy used by (Hayes *et al.*, 1998; Hayes and Meade, 1999) or Klimuk *et al.*, (2000) not the hypersensitivity test described by (Gad *et al.*, 1986; Gad, 1994). An increase in ear thickness of over twenty percent has been used as a positive result (Gad *et al.*, 1986)

Baseline mouse ear thickness of both right and left ears was measured using a micrometer (Mitutoyo, Tokyo, Japan). Then 20 µl of DMSO vehicle was applied to the dorsal and ventral surfaces of left ear pinna using a pipette (Gilson, Middleton, USA) and plastic disposable tip. DMSO (20 µl) containing toxin was applied to the right ear similarly. Both ears were measured hourly for the first eight hours and then daily for at least 14 days. Twenty-four hours after (±1 hour) after the treatment was taken as the measure of ear swelling. Percent ear swelling was calculated as mean

post-treatment ear thickness minus pre-treatment ear thickness divided by pre-treatment ear thickness times one hundred (Figure 4.1). Left ear was used to control for the vehicle. DAT, LA and a 50:50 mix of LA and DAT were added to mice ears in quantities 0.8, 1.2 1.6, 2.4 and 3.2 μg (n = 6 per dose).

$$\% \text{ increase in ear thickness} = \frac{\text{post-treatment ear thickness - pre-treatment ear thickness}}{\text{pre-treatment ear thickness}} \times 100$$

Figure 3.1 Calculation of percent increase in ear thickness.

Crude extracts of *Lyngbya* containing either LA, DAT in the range 0-3.5 μg or neither toxin were applied to mouse ears (n = 3 for each dose). Measurements were conducted as above.

3.3.4 Statistical Analysis

Student's *t*-test was used to examine statistical difference between pre- and post-treatment ear thickness. A graph of toxin dose applied versus percent increase in ear thickness was produced. The sigmoid nature of the curve was noted and the simple sigmoid equation $y = a / \{1 + \exp(-(x-b) / c)\}$ was fitted using Sigma Plot (Jandel Scientific Software, Chicago, USA). Comparison of sigmoid curves was made using an adaptation of Osborne and McNeill (2001). The *b* value of the equation represents the point where half of the maximal response is reached and was used to compare the toxicities of LA, DAT and the LA:DAT mix (1:1). The *b* value occurs on the central linear part of the curve. Comparisons between *b* values were made using Student's *t*-test. $P < 0.05$ was considered significant.

3.3.5 Histopathology

Mice were treated with 3.5 μg toxin (LA, DAT or a 1:1 mixture of LA and DAT) dissolved in 20 μl DMSO, on ventral and dorsal surfaces of the pinna of the right ear. The left ear was treated with 20 μl DMSO. Mice were sacrificed, using CO_2, at 15 minute intervals for two and a half hours (ten mice per treatment). Ears were removed and placed in 10% buffered formalin. Ear thickness was measured prior to sacrifice using a micrometer as above. Slides were made of ear tissue after processing by Veterinary Pathology at the University of Queensland, and stained with haemotoxylin and eosin. Photomicrographs were made using an Olympus zoom stereo microscope (SZ4045TR) with an Olympus BH-2 exposure control unit (Tokyo, Japan) using Kodak "Max" 400 asa film.

3.4 Results -Toxicology

3.4.1 Mouse Ear Swelling Test

There were no toxin related deaths of mice during the trial period. Six mice were euthanised after five days of the trial and three mice after 6 days, as they appeared to be in discomfort. All other mice appeared normal throughout the study apart from erythema and oedema associated with irritancy. All other mouse ears returned to normal thickness. No significant variations in mouse weight were noted for either mice tested with purified toxin or crude extracts (Figure 4.2).

Application of higher doses of purified toxin led to an increase in ear thickness (Figure 3.3). Both LA, DAT and 1:1 mix of LA and DAT (LADAT) elicited similar responses at each dose. Sigmoid curves were fitted to data using the simple sigmoid equation $y = a / \{1 + \exp(-(x-b) / c)\}$ (Figure 3.4). No significant difference was found between half maximal response (b value) for either LA, DAT of LADAT (Table 3.1).

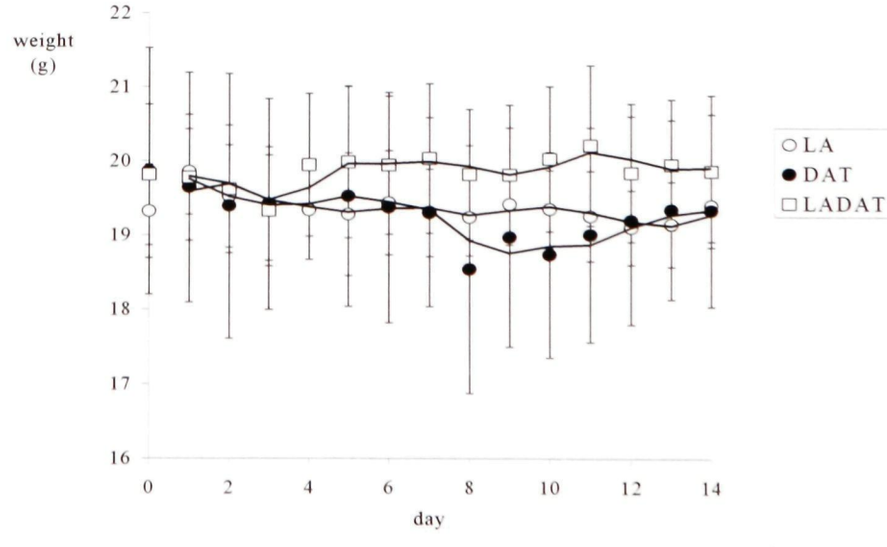


Figure 3.2 Average weights of mice with 3.2 µg of purified toxin applied to ears over a 14 day period.

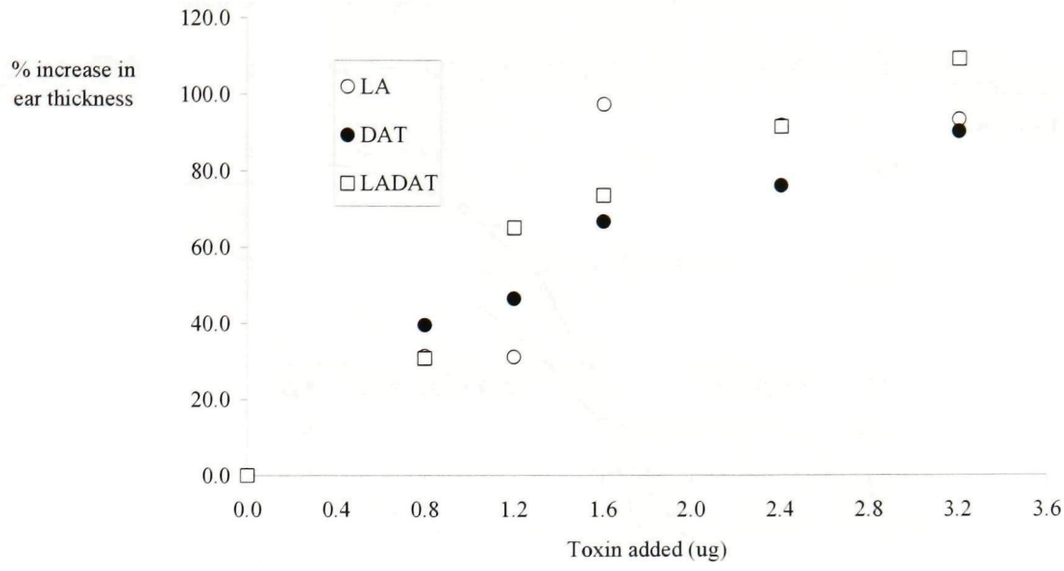


Figure 3.3 Effect of toxin dose on percent increase in ear thickness.

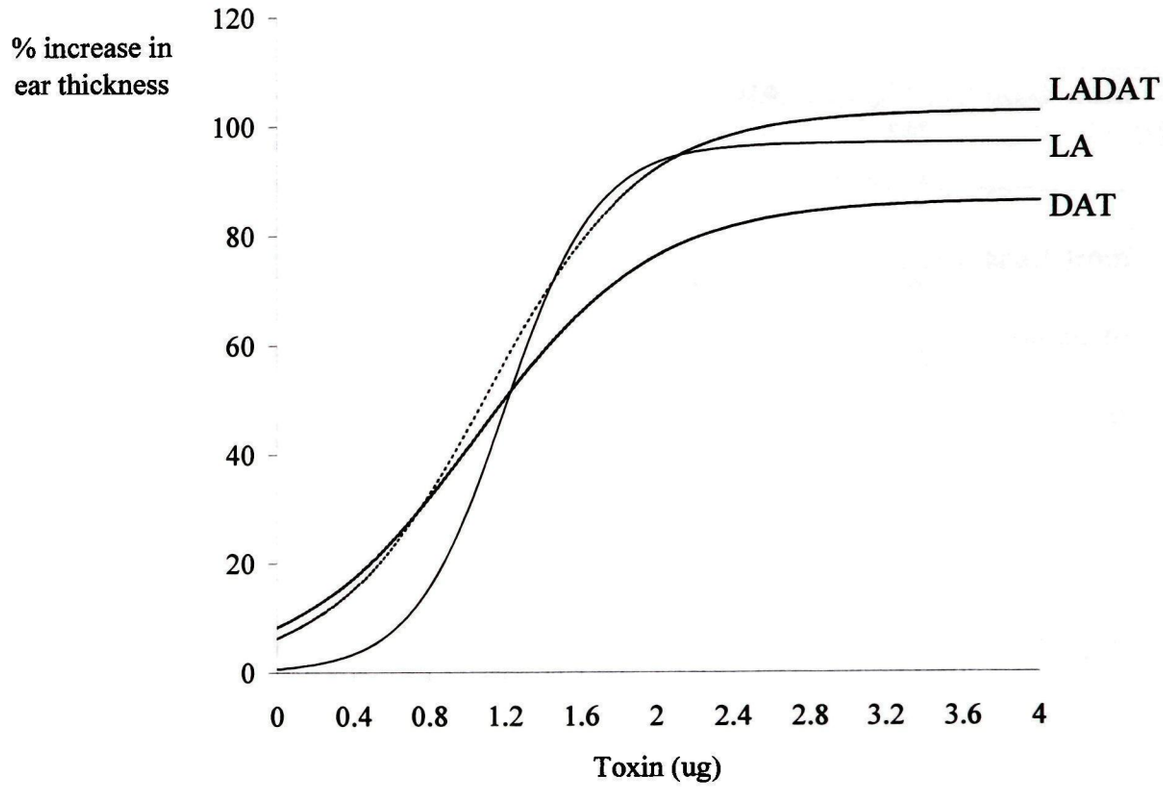


Figure 3.4 Sigmoid curves of toxins applied to mouse ears.

Table 3.1 Amount of toxin (μg) required for half maximal increase in ear thickness. Numbers lacking common superscript in a single column differ, $p<0.05$, Student's *t* test.

	toxin (μg)	std err	r^2 sigmoid curve
LA	1.20^a	0.16	0.90
DAT	1.04^a	0.15	0.96
LADAT	1.10^a	0.12	0.97

3.4.2 Crude Extracts of *Lyngbya*

Crude extracts were produced from samples of *Lyngbya* obtained from Moreton Bay, Florida and Hawaii.

Table 3.2 Crude extracts of *Lyngbya* used in this study.

ID	date collected	location
07c		Honokowi, West Maui
20	25-Feb-00	Bongaree, Deception Bay
12	28-Feb-00	Bongaree, Deception Bay
15	15-Mar-00	Amity Banks
7	14-Mar-00	Amity Banks
16	23-Jun-00	Amity Banks
97	12-Jun-00	Weeki Wakee, Florida
17	05-Apr-00	Pebble Beach, Deception Bay
14	14-Apr-00	Godwin Beach, Deception Bay

Addition of crude extracts containing known concentrations of LA to mouse ears elicited responses greater than with purified LA. Samples containing no LA also elicited a response, although only sample 17 elicited a response greater than 20 percent.

When percent increases in ear thickness for crude extracts containing LA were plotted against the sigmoid curve for purified LA it was found they were above the standard curve (Figure 3.5). These crude extracts caused a greater thickening in mouse ear per unit LA than the purified LA. When crude extracts containing DAT were similarly plotted with the curve for purified DAT there was a closer fit, on or near the curve (Figure 3.6). The effect of crude extracts of *Lyngbya* with no LA or DAT present is also recorded on each graph (Figure 3.5 and Figure 3.6). Control ears (DMSO only) all returned to their previous thickness within 24 hours.

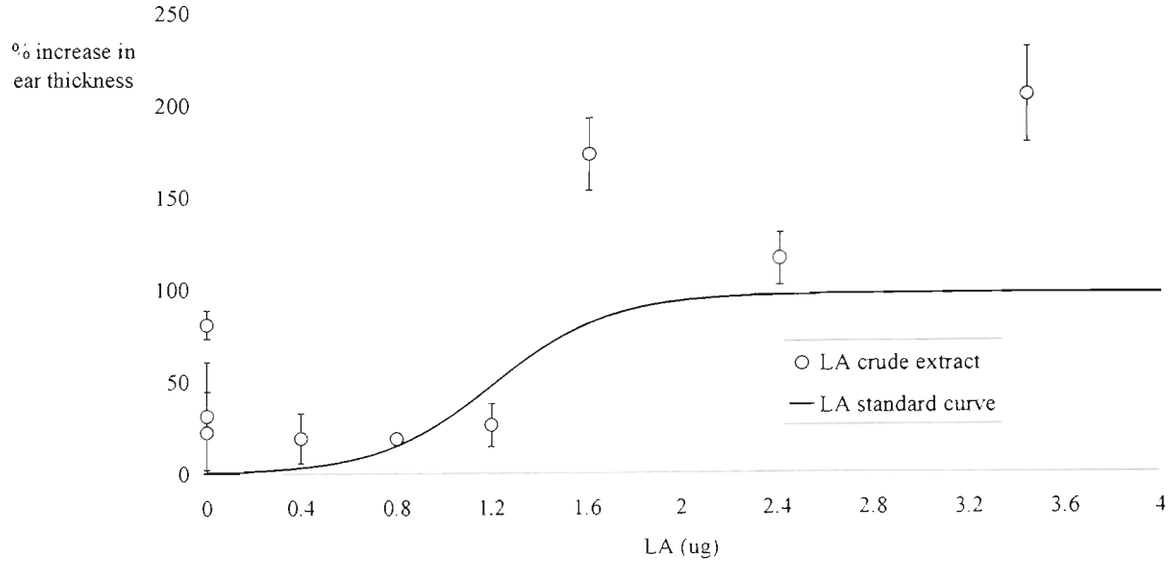


Figure 3.5 Percent increase in ear thickness using crude extracts of *Lyngbya* containing a known concentration of LA in relation to standard sigmoid curve fitted to concentrations of purified LA.

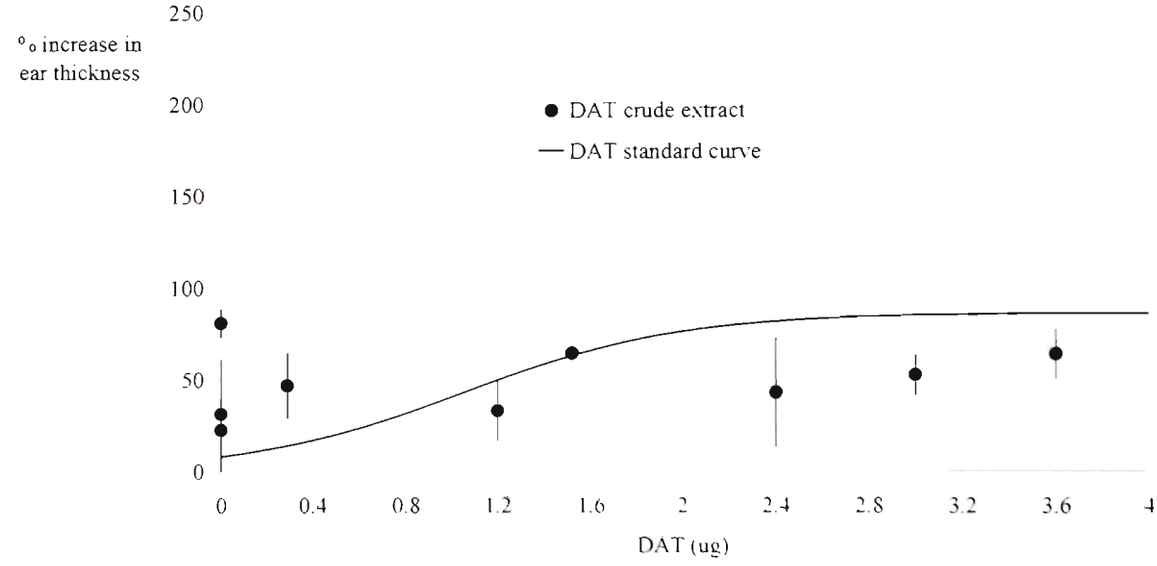


Figure 3.6 Percent increase in ear thickness using crude extracts of *Lyngbya* containing a known concentration of DAT in relation to standard sigmoid curve fitted to concentrations of purified DAT.

As different amounts of crude extract were being added to each ear, a graph of amount of *Lyngbya* added to each ear was plotted against percent increase in ear

thickness. There was no relationship between the amount of crude extract of *Lyngbya* added and increases in ear thickness (Figure 3.7).

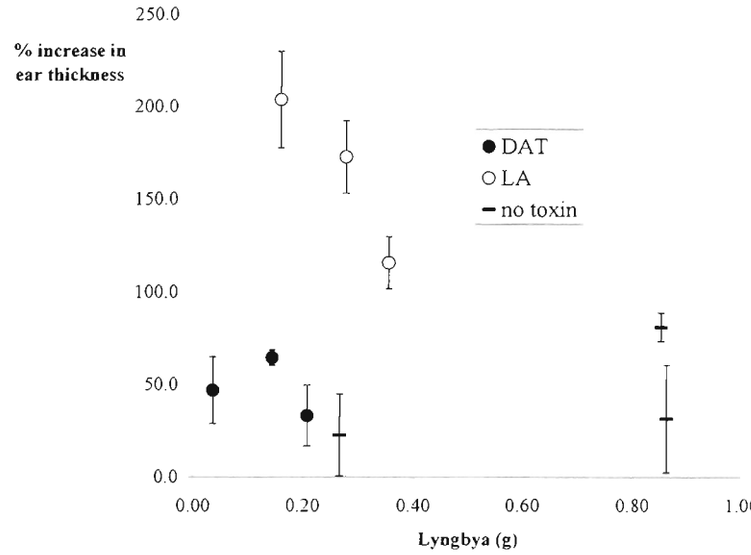


Figure 3.7 Lack of relationship between the LA and DAT contents of *Lyngbya* crude extract applied to ear and the percent increase in ear thickness.

It was noted that maximal ear swelling occurred at different times for purified toxins and crude extracts (Figure 3.8). For the purified toxins maximal ear swelling occurred between 2 hours and 2 days. Crude extracts of *Lyngbya* occurred later, between one and five days. Crude extracts were significantly different from the purified toxins or the average of all purified samples (Student's *t* test, $p < 0.05$) in terms of the number of days until maximum ear swelling was reached.

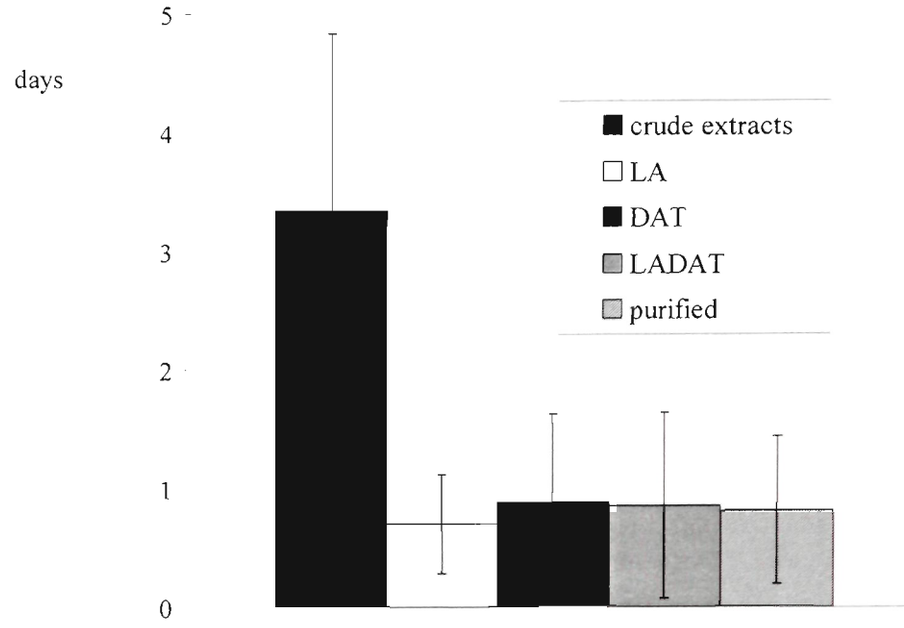


Figure 3.8 Numbers of days until maximal ear swelling was reached.

3.4.3 Other Potential Toxins

As *L. majuscula* has had over 100 chemical species isolated from it, many of which have been shown to be biologically active, it was decided to examine for other toxins. The freshwater species from the same genus, *Lyngbya wollei* has been shown to contain saxitoxins (PSPs) (Carmichael *et al.*, 1997; Onodera *et al.*, 1997). The samples used in crude extracts were examined for the presence of saxitoxins. These included samples 12, 15, 16, 17, 97, and 7. None of these samples had saxitoxins (detection limit 5 μg/L). Other samples from Moreton Bay and Hawaii did not contain saxitoxins.

In addition it was decided to examine for malyngamide H (Orjala *et al.*, 1995a), malyngamide A (Cardellina *et al.*, 1978), microcolin A (Koehn *et al.*, 1992), kalkipyrone (Graber and Gerwick, 1998), bamamide (Orjala and Gerwick, 1996) and antillatoxin (Orjala *et al.*, 1995b). None of these toxins were found in sample "15"

(Amity Banks 15/3/00, LA 21.6 mg/kg). A sample of *L. wollei* from Crystal River/Keys Bay, Florida was found to contain saxitoxins (36 mg/kg).

3.4.4 Histopathogy

A greater than 20% increase in ear thickness occurred after only 15 minutes, with a single dose of LA, DAT or LADAT at 3.2 μg per ear (Figure 3.9). Ear thickness remained constant until 90 minutes (seen as a decrease in thickness of control ear rises) (Figure 3.9). At 60 minutes DMSO began to cause an ear thickening and reached maximum at 75 minutes (Figure 3.10). Ear thickness increased again after 90 minutes for mouse ears treated with toxins (as the effect of DMSO reached a plateau).

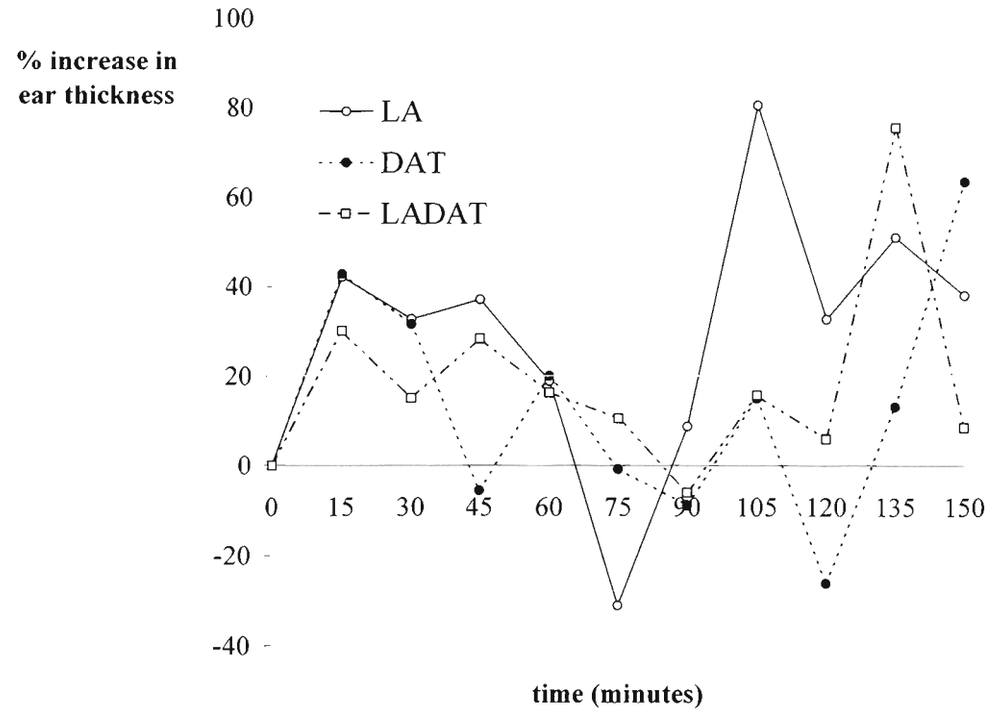


Figure 3.9 Percentage increases in ear thickness with 3.2 μg of LA, DAT or LADAT added. One mouse per treatment.

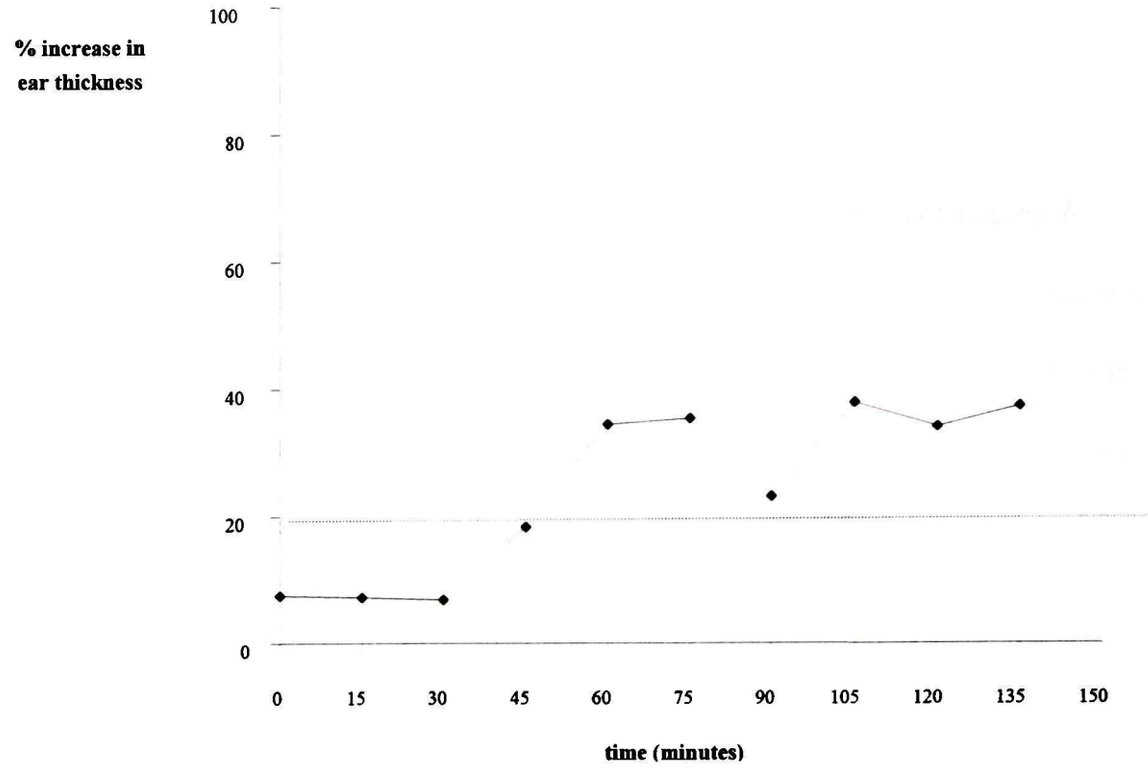


Figure 3.10 Averaged percent increase in control ear thickness (n=3) (DMSO only).

Test ears (LA, DAT or LA+DAT, 3.2 μg) sampled 15 minutes after application have plasma extravasation into the lamina propria. No or minimal infiltration occurred in ears treated with DMSO (control). These increases mirror the differential percent increase in ear thickness between test and control mouse ears. Vacuolisation occurred in the epithelial layer of both test and control ears at 15 minutes and appeared to recede by 150 minutes (Figure 3.18 and Figure 3.19).

At 150 minutes oedema has occurred in both test and control ears (arrowed in Figure 3.12). Oedema was greater overall in test ears. Test ears also have infiltration of neutrophils (Figure 3.14, 3.15 and 3.16). Again the percent increase in ear thicknesses were mirrored in the extravastion in the lamina propria, with greater influxes in test ears.

Table 3.3 Differences in histology at 15 and 150 minutes after dosing.

	Effect at two times since treating	
treatment	**15 minutes**	**150 minutes**
3.2 μg LA	oedema	oedema, neutrophils
control	-	oedema
3.2 μg DAT	oedema	oedema, neutrophils
control	-	oedema
3.2 μg LADAT	oedema	oedema, neutrophils
control	-	oedema

The number of polymorphonuclear leukocytes (neutrophils) seen in mouse ears increased as time since application of toxin increased. Examining slides of mouse ears from different times, the number of neutrophils increased with all toxins except perhaps between 60 minutes and 105 minutes with LA+DAT added. Oedema was greater at 150 minutes than 15 minutes for the test ears. Test ears had greater oedema than control ears.

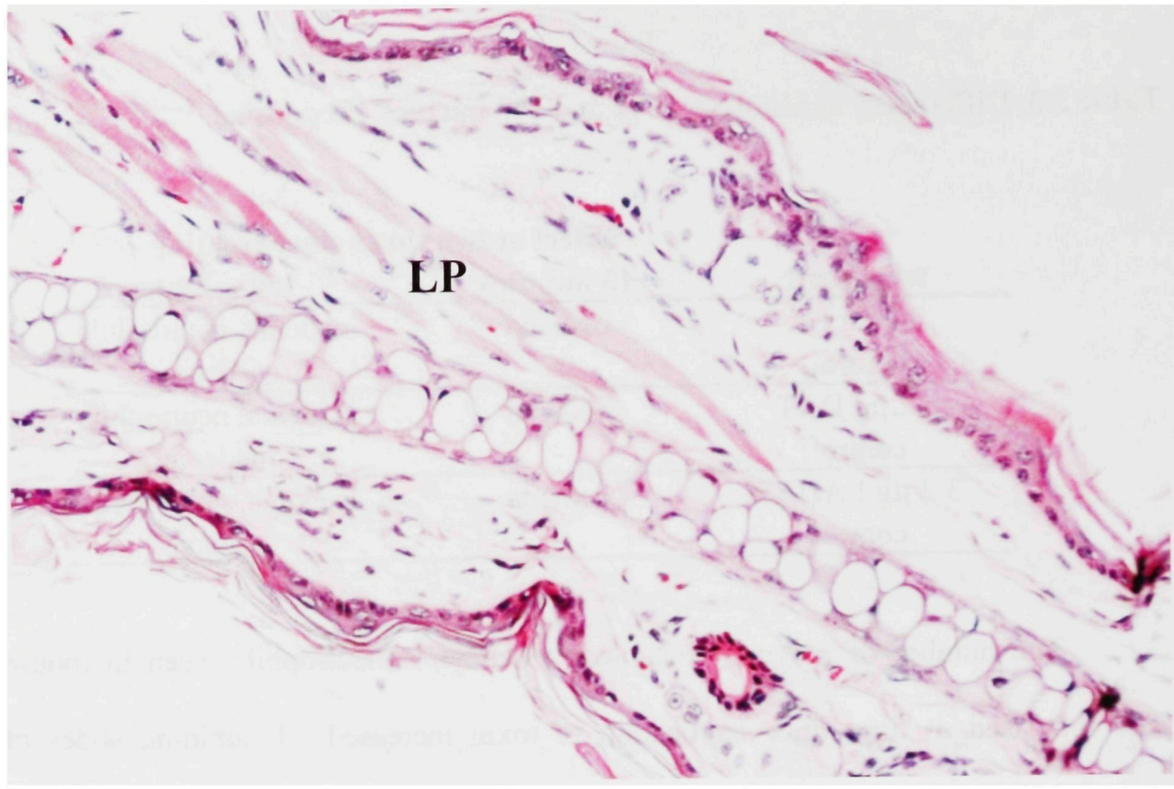


Figure 3.11 Photomicrograph of mouse ear treated with negative control (DMSO) 15 minutes after application. (H & E, LM, x 200). LP: lamina propria. Little or no oedema in LP.

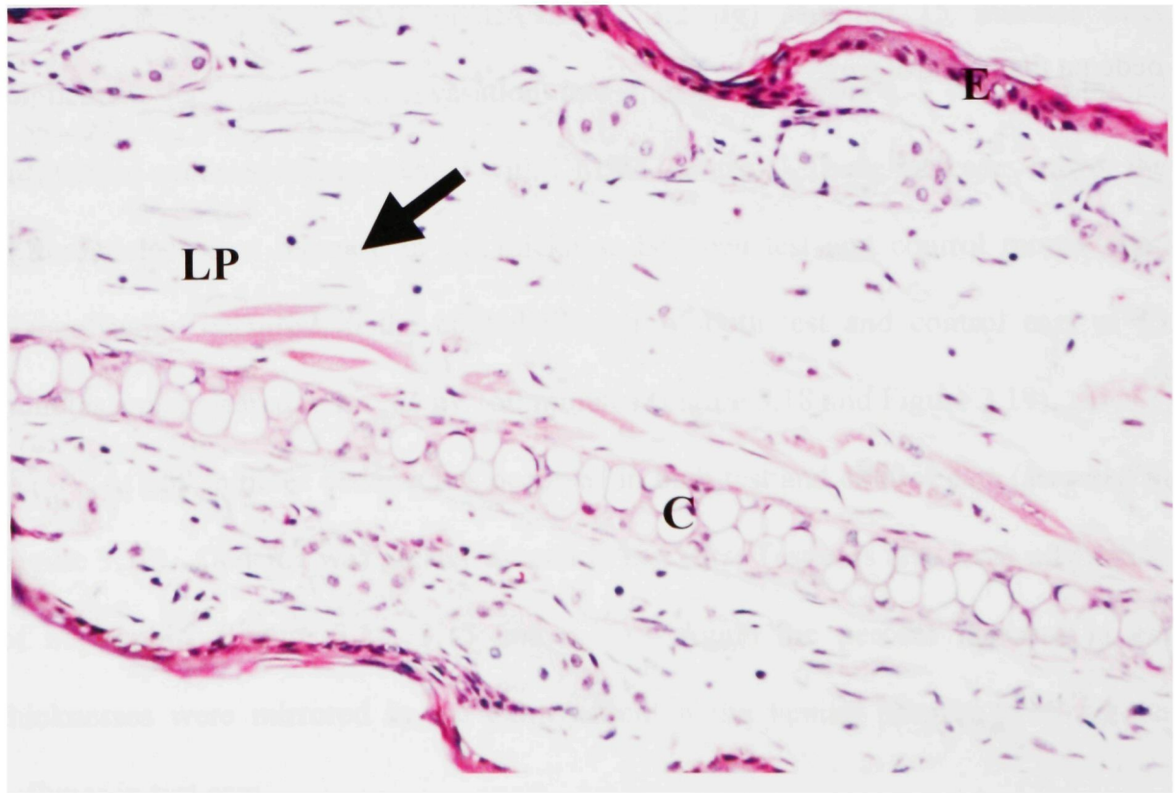


Figure 3.12 Negative control ear (DMSO) 150 minutes after application (H & E, LM, x 200). E epithelial layer, C: connective tissue. Slight oedema of the LP (arrowed).

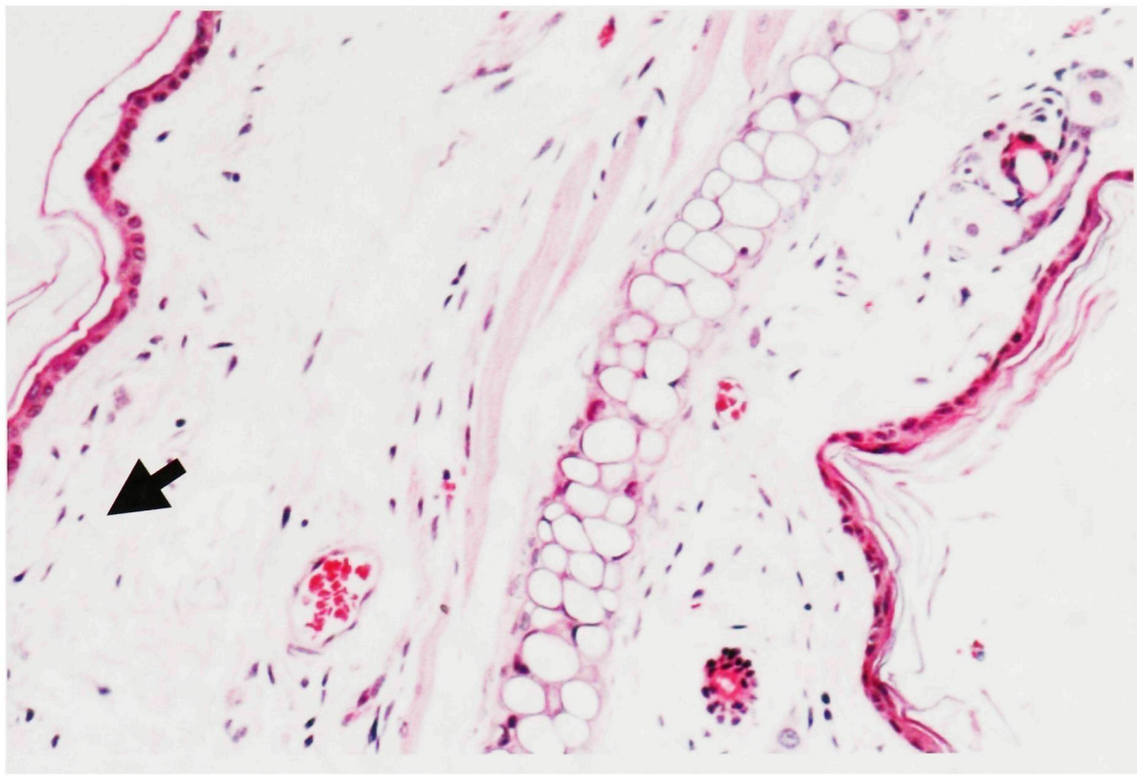


Figure 3.13 LA test (3.2 μg) 15 minutes after application (H & E, LM, x 200). Slight oedema of LP (arrowed).

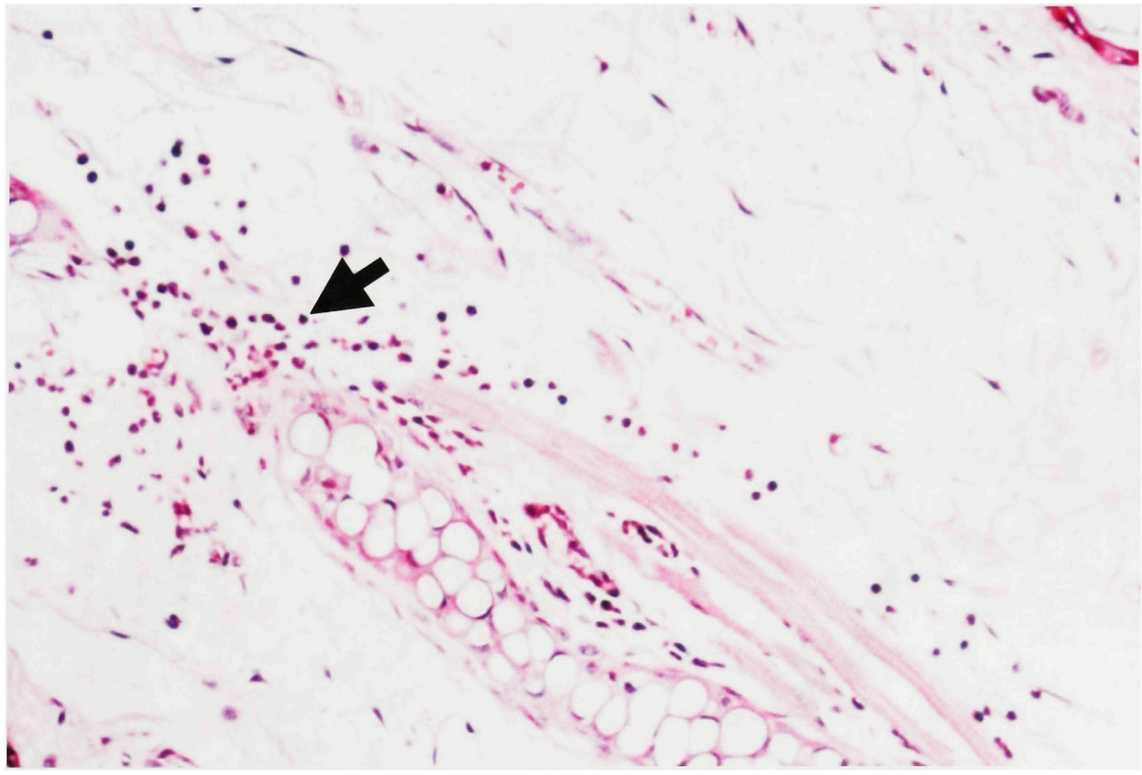


Figure 3.14 LA test (3.2 μg) 150 minutes after application (H & E, LM, x 200). More extensive oedema of LP and influx of neutrophils (arrowed).

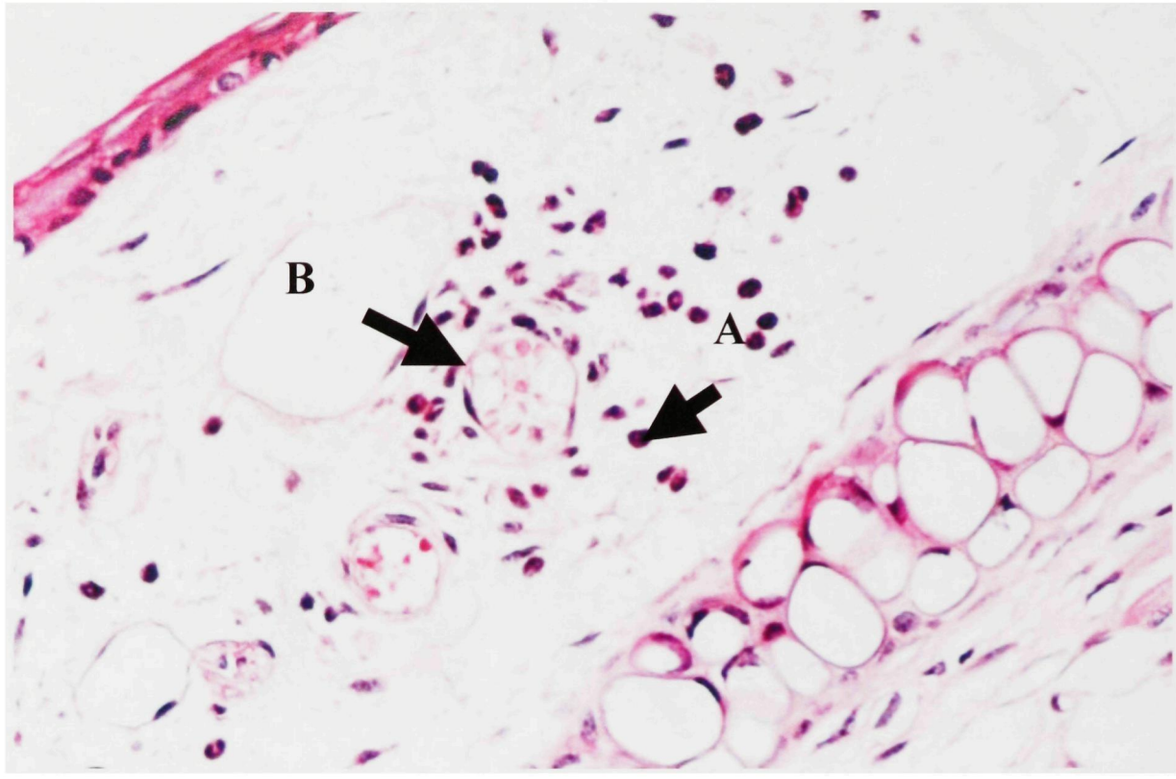


Figure 3.15 Influx of neutrophils. DAT test (3.2 µg) 150 minutes (H & E, LM, x 400). A: Neutrophils, B: neutrophils moving through the wall of blood vessel.

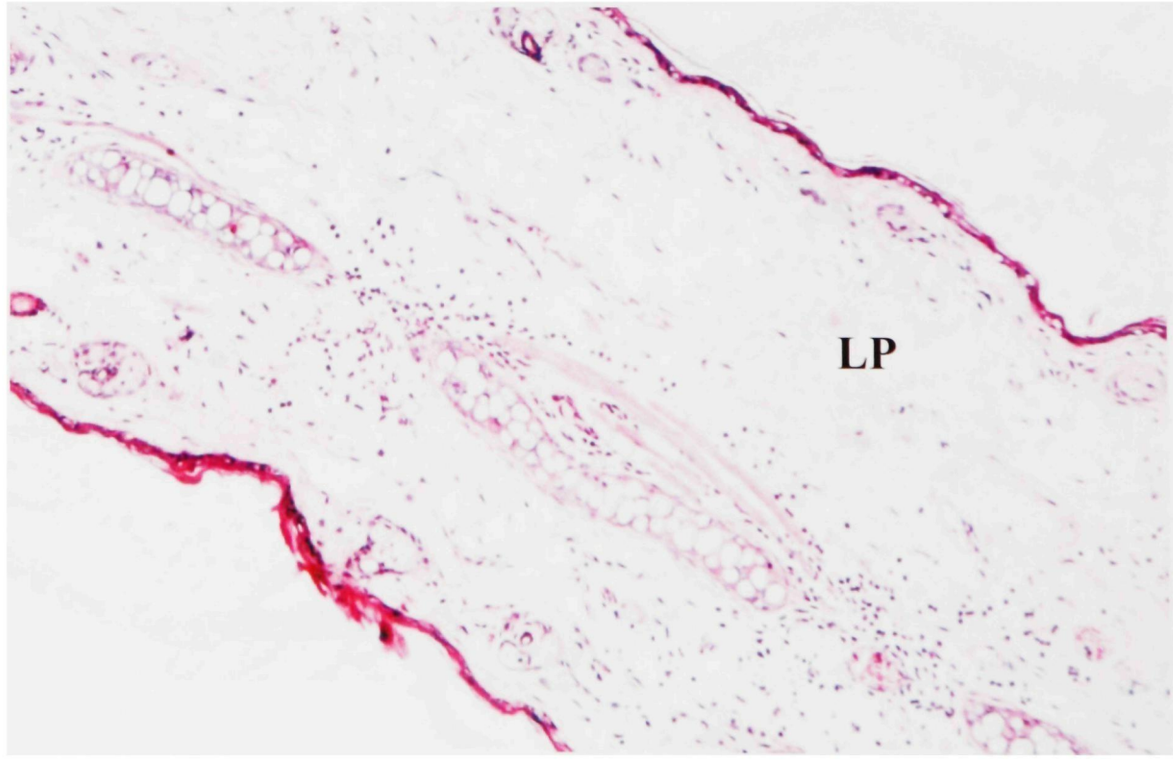


Figure 3.16 Influx of neutrophils (arrowed). LA test (3.2 µg) 150 minutes (H & E, LM, x 100). Oedema in LP.

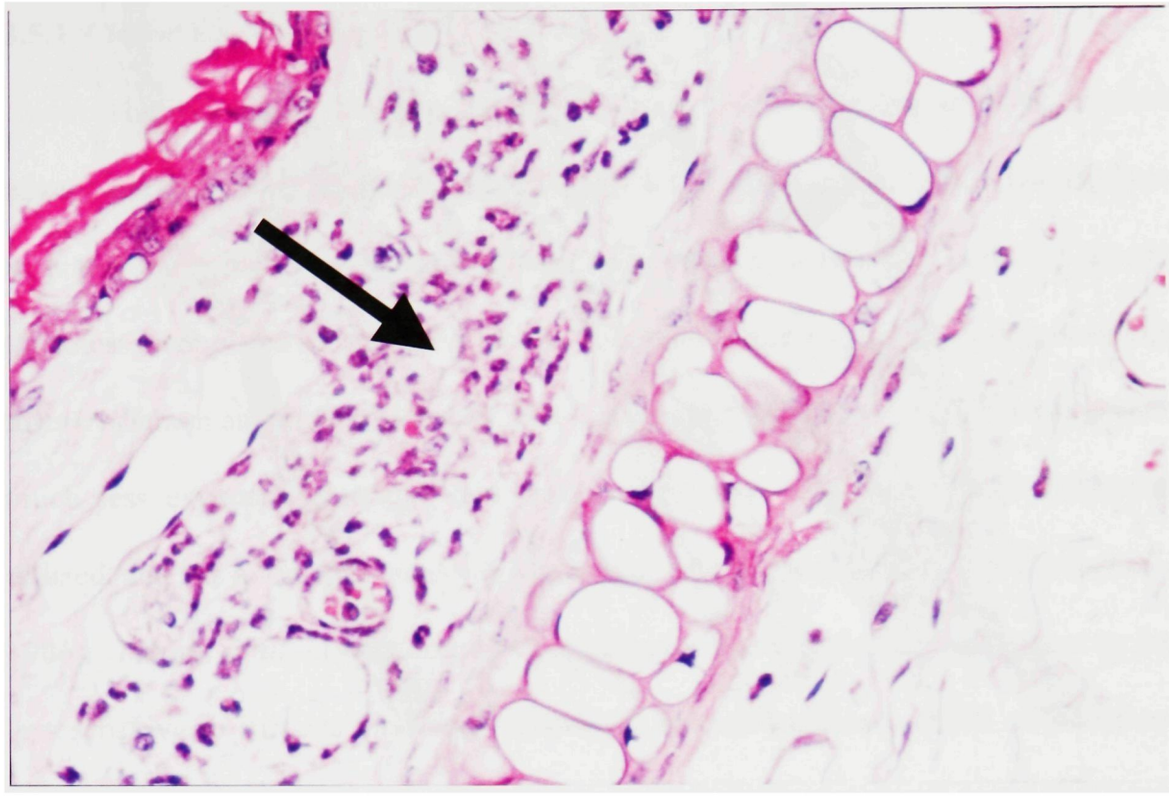


Figure 3.17 Influx of neutrophils (arrowed) DAT test (3.2 μg) after 150 minutes (H & E, LM, x 400).

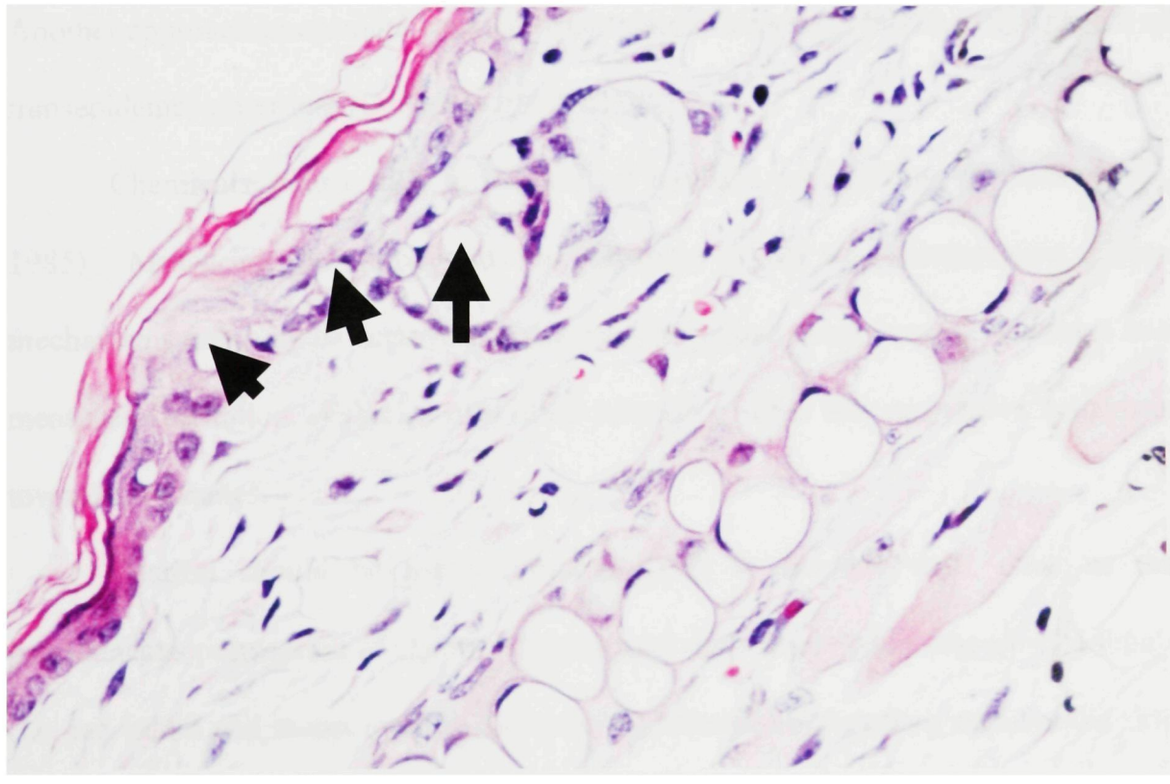


Figure 3.18 Vacuolisation occurred in epithelial layer after 15 minutes (DAT control) (H & E, LM, x 400). Vacuoles arrowed.

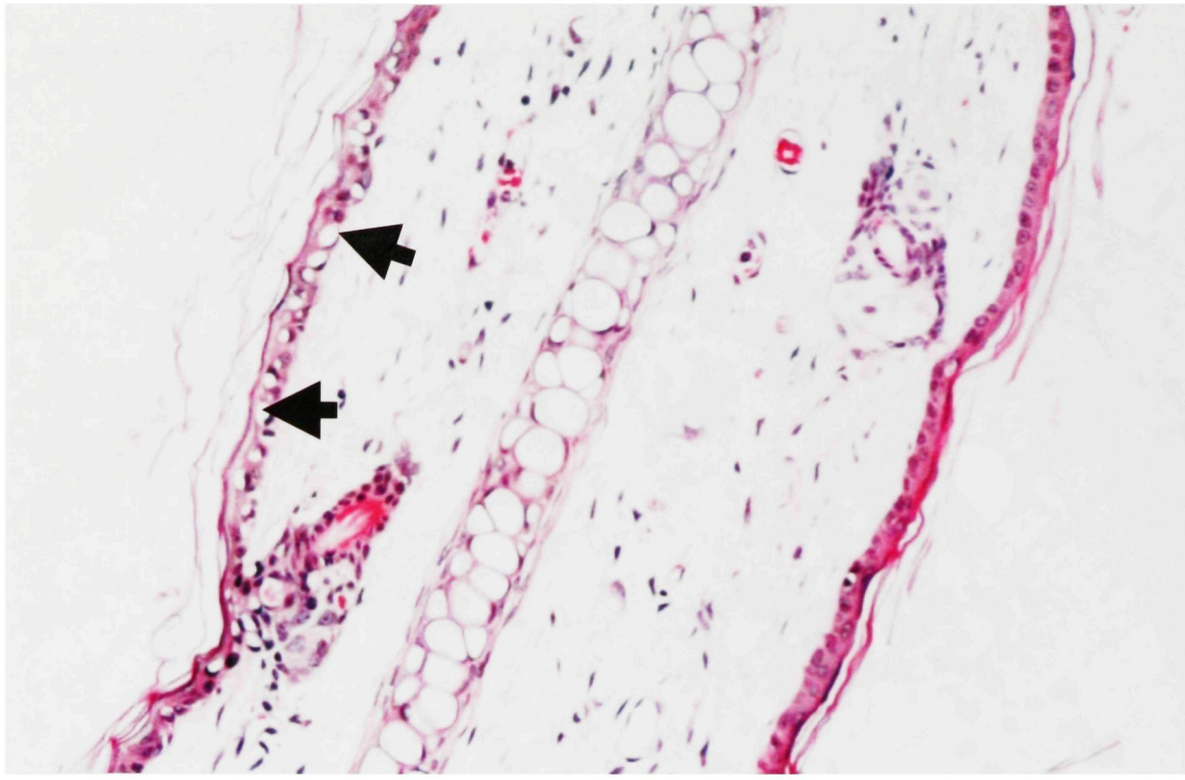


Figure 3.19 Vacuolisation occurred in epithelial layer after 15 minutes (DAT test) ((H & E, LM, x 200).

3.5 Discussion - Toxicology

3.5.1 Mouse Ear Swelling Test

The mouse ear swelling test (MEST) was chosen for use as it has several advantages over other available tests. The MEST has been noted to be advantageous as a test for irritant contact dermatology as it gives a quantitative endpoint of ear thickness as opposed to subjective scores of erythema and oedema used in guinea pig tests (Asherson and Ptak, 1968; Gad, 1994; Hayes and Meade, 1999). This test is also much less expensive, requires less space, duration is shorter, less test substance utilised, and has a low false negative rate as well as a no false positive rate (Gad *et al.*, 1986). Patrick *et al.* (1985) do point out however that the mouse does appear to be less sensitive to irritants than other laboratory animals and man, which may lead to false negatives or difficulties in extrapolating results. However as LA and DAT are strong irritants, the MEST is suitable as it measures oedema and cell infiltration. Another approach to examine contact irritant dermatitis would be the measurement of transepidemal water loss (Loffler *et al.*, 2001).

Chemicals induce skin irritations by multiple mechanisms (Patrick *et al.*, 1985). Measuring the endpoint of ear thickness allows the incorporation of any mechanisms that may be occurring which may be missed using *in vitro* bioassays as a measure of irritation. Females were used because of their less aggressive behaviour toward cage mates.

Caution should be used if extrapolating to humans from mice as the percutaneous permeation of chemicals across the skins of common laboratory animals (i.e. rat, rabbit and mouse) was found to be significantly higher than with human skin (Wester and Maibach, 1987). Lipid component and organisation of the stratum corneum appear to be the factors most likely to influence differences (Bond and

Barry, 1988; Suber *et al.*, 1991). This has been shown to be the case with LA, with 23.0 percent penetrating over one hour for guinea pig and 6.2 percent of dose for human skin (Stafford *et al.*, 1992). It should be noted that the ability of *L. majuscula* to cause contact hypersensitivity reactions which may mimic the *in vivo* response to dermal toxins has not been assessed here. Other groups have suggested that tumour promoters may in fact suppress the development of contact hypersensitivity (Czernieki *et al.*, 1988). Other cyanobacteria have been found to inhibit anaphylaxis (Yang *et al.*, 1997). Work by Grauer and Arnold (1961) suggested that *L. majuscula* did not cause hypersensitivity.

3.5.2 Vehicle

Dimethyl sulphoxide (DMSO) was used as a vehicle because it is able to dissolve a wide variety of compounds of differing lipophilicities. In order to ensure that the greatest amounts and varieties of compounds were extracted from the *Lyngbya* material, it was decided to use this solvent as a vehicle. DMSO has previously been used successfully as a vehicle in MEST (Howell *et al.*, 2000). Fears that increased absorption may occur using a strongly lipophilic vehicle as DMSO were allayed by the work of Patrick *et al.* (1985) which showed that differences in time courses of the response to chemical irritants were not altered by varying the rate of absorption. Despite being described as an anti-inflammatory agent it did induce some swelling in the mouse ear, this had receded after approximately 8 hrs, with 24 hours the point at which ear thickness was measured in these trials. DMSO has a variety of actions in mammals including membrane penetration, anti-inflammation, vasodilatation, bacteriostasis, effects collagen, nerve blockade, muscle relaxation, non-specific

enhancement of resistance, alterations of activity for concomitantly administered drugs, cholinesterase inhibition and diuresis (Wood and Wood, 1975).

3.5.3 Weight Loss

No appreciable weight loss was noted with mice in this experiment. Previously weight loss has been noted in animals undergoing MEST. Application of 3% dicyclohexylcarbodiimide (DCC) to mouse ears caused significant weight loss while the structurally related diisopropylcarbodiimide (DIC) (0.06-20%) did not (Hayes *et al.*, 1998).

3.5.4 Similarities in Effects of LA and DAT

No quantitative difference was found between LA and DAT in their ability to cause a thickening of mouse ears on dermal application (Figure 3.4). As LA and DAT act via the same receptor system (Fujiki *et al.*, 1981; Fujiki *et al.*, 1984b) and many biological activities and potencies are similar, it may be presumed that the effects may be similar. But as skin irritation is made up of multiple mechanisms it was still unclear if these two toxins would produce the similar degrees of irritation. LA and DAT biological activities have been explored using both *in vivo* and *in vitro* techniques (Table 3.4). Some potencies and activities of LA appear to be similar to DAT, whilst others are different.

Other measurements of the irritating nature of LA and DAT have found similarities. Fujiki *et al.* (1983b) measured the dose where 50 percent of mice had reddened ears 24 hours after application using the irritant test on mouse ear after Hecker (1971). Fujiki *et al.* (1983b) found LA required 0.011 nmole/ear while AT and DAT required 0.005 nmole/ear. Other tumour promoters such as TPA, teleocidin

and dihydroteleocidin B were in a similar range. Fujiki *et al.* (1990) later reported similar irritancy at 0.1nmol for LA and DAT. Fujiki *et al.* (1990) reported similar irritancy at 0.1nmol for LA and DAT, but AT was greater while bromoaplysiatoxin and oscillatoxin A were lower and dibromoaplysiatoxin caused no irritation.

Other biological activities of LA and DAT have also been found to occur at similar potencies. The size of effects associated with the tumour-promoting activities of LA and DAT are similar. Induction of ornithine decarboxylase (ODC) was similar between LA and DAT. With a dose of 11 nmol of LA, ODC was induced 3.31 nmol CO_2/mg protein/30 min while 3 nmol DAT induced ODC 5.52 nmol/CO_2/mg protein/30 min (Suganuma, *et al.*, 1984). The ED_{50} of LA to inhibit specific binding of ^{3}H-TPA was 8.0 nM while DAT was 6.8 nM (Suganuma *et al.*, 1984). Similarly the *in vitro* activation of protein kinase C by LA was 3.3 pmol/min/1.0 μg compound while DAT was 4.1 pmol/min/1.0 μg compound (Fujiki *et al.*, 1990). *In vivo*, the number of tumour-bearing mice in week 30 after application of 6.9 nmol of LA was 86.6 percent while with 4 nmol DAT, 71.4 percent were tumour bearing (Fujiki *et al.*, 1982b; Fujiki *et al.*, 1990). Horowitz *et al.* (1983) found no differences in the ability of a mixture of teleocidin A and B (93:7) and DAT to stimulate choline or arachidonic acid release in rat or mouse embryo cells.

As well as similarities in the potencies and activities between LA and DAT some differences have been found. The quantity of DAT required for 50% adhesion of HL-60 cells was found to be 180 ng/ml, while LA and AT required doses far less at 7.0 ng/ml and 2.0 ng/ml, respectively (Fujiki *et al.*, 1981). Similarly the ED_{30} of phagocytosis for DAT was 100 ng/ml while LA and AT was 2.5 ng/ml and 1.7 ng/ml, respectively (Fujiki *et al.*, 1981; Nakayasu *et al.*, 1981). The ED_{50} for the activation

of calcium-activated, phospholipid-dependant protein kinase (protein kinase C) required 40 ng/ml of teleocidin, while DAT required 400 ng/ml (Fujiki *et al.*, 1984b).

Horowitz *et al.* (1983) established several differences *in vitro* in the receptor binding ability of DAT and "teleocidin", a mixture of 93% teleocidin A and 7% teleocidin B. LA is one isomer of teleocidin A (Cardellina *et al.*, 1979). An assay for the specific binding of the phorbol ester, [^{3}H]-phorbol-12,13-dibutyrate to monolayer cultures was used. Teleocidin was applied to rat embryo cell lines (CREF cells) and mouse embryo cell lines (C3H10T½ cells) at 11 nM and 7 nM, respectively. For the same effect 94 nM and 200 nM for DAT needed to be used. Similarly, differences in ED_{50} for inhibition of [^{125}I]-epidermal growth factor was found with 2.9 nM and 1.8 nM for teleocidin, again on CREF and C3H10T½ cells, while DAT was higher at 55 nM and 42 nM. Stimulation of choline release and arachidonic acid was similar for teleocidin and DAT in both cell lines. Interestingly, AT followed the pattern set by the mixture of teleocidins, not DAT (Horowitz *et al.*, 1983).

The experiments assessing the combined effect of LA and DAT was additive in nature as witnessed by the sum of DAT and LA having a similar half maximal dose value as DAT and LA. Synergism was not observed. Experiments with other cyanobacteria have shown similar additive effects amongst toxins contained in the same organism. Microcystin-LR, -YR and –RR showed a simple additive effect (Fastner *et al.*, 1995), suggesting no synergy and a simple concentration additivity model (Faust *et al.*, 1994). Ohuchi *et al.* (1986) found that TPA-type tumour promoters, including AT and TPA, did not act synergistically in respect to the release of histamine from isolated rat peritoneal mast cells. However, synergism did occur between the non-TPA-type tumour promoter thapsigargin and AT and teleocidin (TPA-type). Suganuma *et al.* (1993) also found no synergistic relationship between

teleocidin and okadaic acid in CD-1 mouse skin. The laxaphycins extracted from *L. majuscula* have been found to exhibit biological synergism when tested for antifungal and cytotoxic effects (Bonnard *et al.*, 1997).

Table 3.4 Biochemical effects of LA and DAT.

Author	assay	Comparison of LA and DAT
Fujiki *et al.* (1981)	induce ornithine decarboxylase dorsal mouse skin	similar in vivo
Horowitz *et al.* (1983)	stimulation aracadonic acid release	
Sakamoto *et al.* (1981)	stimulation choline turnover	
Sakamoto *et al.* (1981)	prostoglandin production	similar*
Horowitz *et al.* (1983)	receptor binding and phospholipid metabolism	similar#
Goldstein *et al.* (1981)	superoxide anion radical production	
Horowitz *et al.* (1983)	inhibition of epidermal growth factor binding	
Eliasson *et al.* (1983)	induction Epstein-Barr virus (EBV)	
Eliasson *et al.* (1983)	enhanced EBV-induced transformation	
Nakayasu *et al.* (1981)	induction of terminal differentiation HL-60 cells	
Fujiki *et al.* (1981)	adhesion HL-60 cells	DAT 100x less in vitro
Fisher *et al.* (1982)	aggregation lymphoblastoid cells	
Fujiki *et al.* (1981)	inhibition of terminal differentiation leukemia cells	DAT 100x less in vitro
Fujiki *et al.* (1982)	aggregation of NL-3 cells	
Fisher *et al.* (1982)	stimulation 2-deoxyglucoses transport	
Fisher *et al.* (1982)	enhanced transformation by adeno-virus	
Fisher *et al.* (1982)	enhanced cloning efficiency of transformed cells	
Fisher *et al.* (1982)	inhibition melanogenesis in B16 cells	
Fisher *et al.* (1982)	inhibition myogenesis	
Keisari *et al.* (1985)	stimulation monocytes	
Ramos *et al.* (1984)	enhanced function of lymphocytes	
Fujiki *et al.* (1984a)	co-carcinogens (papillomas)	
Moore *et al.* (1986)	bind to high affinity receptor in mouse skin	
Fujiki *et al.* (1984b)	binding to protein kinase C	
Arcoleo and Weinstein (1985)	activation of protein kinase C (no Ca^{2+})	
*AT not DAT # tribromoaplysiatoxin = 3xBr		

3.5.5 Differential Effects between Crude Extracts and Purified Toxins

The increased potency of crude extracts has been noted for several cyanobacterial toxins (Jungmann and Benndorf, 1994; Bury *et al.*, 1995; Fastner *et al.*, 1995; Oberemm *et al.*, 1997; Kyselkova and Marsalek, 2000; Pietsch *et al.*, 2001). Suggested causes for differences include the presence of a factor leading to increased action via the same mechanism. Alternatively, it may be due to other chemicals present working via a separate mechanism to enhance the ear swelling.

Jungmann and Benndorf (1994) found no correlation between microcystin concentration of *Microcystis* samples and the ability of these to cause toxicity in *Daphnia pulicaria.* Jungmann (1992) had previously shown that toxicity of water extracts of *Microcystis* to *Daphnia* were high in microcystin-free fractions. The presence of a "*Daphnia*-toxic compound" was therefore proposed, present at comparable levels in natural and laboratory cultures (Jungmann and Benndorf, 1994). Previously (DeMott *et al.*, 1991) had proposed that chemicals were present in *Microcystis* that were more toxic against *Daphnia* than the two microcystins they had isolated from their culture.

Fastner *et al.* (1995) found that the hepatotoxicity exhibited by crude extracts was significantly higher than explained by their content of microcystins. Using a colorimetric assay of rat primary hepatocyte viability, toxicity was found to be two to three times higher than expected from microcystin levels. Tests using CHO-K1 cell lines showed substances possibly leading to unexplained toxicity do not enter the cells readily, and damage to cytoskeleton by microcystins, rapidly taken up by carrier-mediated transport systems, may increase permeability of other substances (Fastner *et al.*, 1995). Differing toxicities were probably not due to different ratios of microcystin sub-species as these have been found to have an additive, not a synergistic effect (Fastner *et al.*, 1995).

Bury *et al.* (1995) found that brown trout exposed to lysed toxic *Microcystis aeruginosa* grew less than those exposed to either lysed non-toxic cyanobacteria or purified microcystin-LR added at equivalent levels. All three treatments inhibited growth more than controls. While short-term exposure (4 hours) had little effect (at 41-68 $\mu g.L^{-1}$) chronic exposure (63 days) resulted in reduced growth rates. Exposure

to toxins led to higher Na^+ influx rates and higher body ion levels, perhaps due to increased levels of stress hormones such as cortisol (Bury *et al.*, 1995).

Examining the toxicity of crude *L. majuscula* extract and the purified toxins barbamide, antillatoxin, malyngamide H and curacin A, the Gerwick group found that not all the toxicity was explained by these four compounds (Gerwick *et al.*, 1994; Orjala *et al.*, 1995a; Orjala *et al.*, 1995b; Orjala and Gerwick, 1996). It was postulated that the selectivity of these compounds to only one class of animal represents independent adaptions by *L. majuscula* to these different grazers. The apparent absence of other antifeedant chemicals in *L. majuscula* collected from Moreton Bay (Pers. Comm. Mary Garson, University of Queensland), combined with the extensive blooms suggests a lack of topdown predation of this cyanobacterium.

Oberemm *et al.* (1997) found aqueous cyanobacterial crude extracts from field samples and batch cultures had greater effect on embryo-larval development of zebrafish (*Danio rerio*) that purified microcystin-LR. The authors suggested that the effects of pure microcystin was much less evident due to a very low rate of uptake. The reasons for the increased toxicity of crude extracts was proposed to be due to increased rates of uptake of toxins, synergistic actions of toxins, or unknown substances increasing toxicity. Alternatively, other toxins may be present, as suggested by (Jungmann, 1992).

A decrease in the activity of phase II detoxification enzyme GST occurred with crude extracts of cyanobacterial bloom material (80% *Microcystis aeruginosa* and 20% *Aphanizomenon flos-aquae*) while purified microcystin (LR and RR) increased their activity (Pietsch *et al.*, 2001). The differential effects of GST activity were noted in the microalgae *Scenedesmus armatus*, the marcrophyte *Ceratophyllum*

dermesum, and zebrafish (*Danio rerio*) eggs. Interestingly, no changes were noted with the invertebrate *Chaoborus crystallinus*.

These results may also be relevant to higher mammals and humans with these groups similarly showing that microcystin-LR was conjugated by human recombinant GST isozymes A1-1, P1-1 and M1-1 (Pflugmacher *et al.*, 2000) as well as rat cytostolic and microsomal GST (Takenaka, 2001).

The reduction in the activity of this enzyme inhibits the ability of an organism to detoxify microcystins, in turn enhancing their effects. The authors postulate that these modulating factors may be in an LPS fraction present in the cyanobacterial bloom sample from cyanobacterial and/or bacterial sources. Little knowledge exists on the biological effects produced by cyanobacterial capsular polysaccharides (Filali-Mouhim and Hours, 1995). LPS is reported by some to reduce the activity of enzymes (Choi and Kim, 1998; Marionnet *et al.*, 1998; Nadai *et al.*, 1998). LPS alone may not induce inflammation but it may reduce the ability of an organism to detoxify any other toxins present. LPS from both *Microcystis* sp. and *Gloeotrichia* sp. reduced activity of GST isoenzyme, including the ability to conjugate with microcystin-LR (Pflugmacher *et al.*, 2000). The effect of microcystins on GST cannot be readily extrapolated to other cyanobacteria with *Spirolina platensis* inducing the activity of GST in the liver, lung, kidney and forestomach, as well as reducing tumour burden in skin tumour studies (Dasgupta *et al.*, 2001). The role of exopolysaccharide producing-cyanobacteria in the production of marine benthic mucilaginous aggregates has been recently disputed, with other organisms, not the primary colonising cyanobacteria, probably having the major contribution (De Philippis *et al.*, 2002).

Tarczynska *et al.* (2001) report that higher toxicity was found with crude extracts of *Microcystis aeruginosa* as opposed to purified toxins using microbiotests

with the protozoan *Spirostomum ambiguum*, the crustacean *Thamnocephalus platyurus* and the cladoceran *Daphnia magna*. Averaged EC_{50} values using commercially available toxicity testing kits also reflected a higher toxicity among crude extracts.

The presence or absence of substances from the crude extracts but not the purified toxins may alter pH. This may in turn change the hydrophobicity of toxins (DeMott *et al.*, 1991) which can effect the bioavailability and hence toxicity (Ward and Codd, 1999).

Other authors have also found crude extracts of *M. aeruginosa* more toxic than purified microcystins, probably due to other toxic factors being present (Kyselkova and Marsalek, 2000). Microcystin does however seem to be involved with toxicity, with good correlation coefficients between both crude fraction and toxin fraction and microcystin concentration for three species tested.

Only crude extracts containing LA increased oedema above levels achieved with pure toxin, while DAT did not. This difference between LA and DAT samples could be due to a variety of reasons. The two most probable reasons are that other toxins were present in the LA samples. Alternatively, a potentiating factor, not necessarily toxic by itself, was present or only was active in the LA sample. As LA and DAT have similar potencies in many of their vast range of biological activities and act via the same receptor, it is probably more likely that anther toxin is present, as has been found with *Microcystis*. A third alternative exists that anti-inflammatory chemicals may exist in the DAT crude extracts. No relationship was found between the amount of crude extract of *Lyngbya* applied to an ear and the percent increase in ear thickness. These results suggest if another toxin is present, it is not ubiquitously present in *Lyngbya* samples, or at least is present in varying levels.

Many toxins have been characterised from *L. majuscula*. Many of these have been shown to have toxicological effects including cytotoxic properties. These are outlined in Table 3.5. Potentially these could add to or augment the effects lyngbyatoxin A, increasing the response if they were present.

Table 3.5 Toxicity data for secondary metabolites of *L. majuscula*.

Name	ichthotoxic LD_{50}	brine shrimp LD_{50}	cytotoxic IC_{50}	other	author
antillatoxin A	0.05ug/ml				Orjala *et al*., (1995)
apratoxin A			0.52nM		Luesch *et al*., (2002)
E-dehydroapratoxin A			37.6nM		Luesch *et al*., (2002)
apratoxin B			21.3nM		Luesch *et al*., (2002)
apratoxin C			1.0nM		Luesch *et al*., (2002)
barbamide	ND	ND		molluscocidal LC_{100}=100ug/ml	Orjala and Gerwick, (1996)
carmabin A			ND		Hooper *et al*., (1988)
carmabin B			ND		Hooper *et al*., (1988)
curacin A		0.003ug/ml	0.0068ug/ml		Gerwick *et al*., (1994)
dolastatin 3			cytotoxic		Mitchell *et al*., 2000)
dragonamide			>1ug/ml		Jimenez *et al*., 2001)
grenadamide		5ug/ml			Sitachitta and Gerwick, (1998)
hermitamide A	19uM	5uM	2.2uM	nil molluscocidal	Tan *et al*., 2000)
hermitamide B	25uM	18uM	5.5uM	nil molluscocidal	Tan *et al*., 2000)
isomalyngamide A				LD crayfish 250ug/kg	Kan *et al*., 2001)
somalyngamide B				LD crayfish 500ug/kg	Kan *et al*., 2001)
kalkipyrone	2ug/ml	1ug/ml			Graber and Gerwick, (1998)
kalkitoxin	700nM	170nM	3.86nM	sea urchin eggs 25nM	Berman *et al*., (1999)
laxaphycin A			synergistic with B		Bournard *et al*., (1997)
laxaphycin B			1.1mM		Bournard *et al*., (1997)
lyngbyabellin A			0.03ug/ml	ip mice 2.4mg/kg	Luesch *et al*., 2000b)
lyngbyabellin B		3.0ppm	0.1ug/ml		Luesch *et al*., 2000a)
lyngbyastatin 1				disrupter microfilaments networks	Harrigan *et al*., (1998)
majusculamide C			cytotoxic		Carter *et al*., (1984)
malyngamide A		0.016ug/ml			Cardellina *et al*., (1978)
malyngamide B		0.016ug/ml			Cardellina *et al*., (1978)
malyngamide H	5ug/ml				Orjala *et al*., (1995)
malyngamide I	<10ug/ml	35ug/ml			Todd and Gerwirck, (1995)
malyngamide J	40ug/ml	18ug/ml			Wu *et al*., (1997)
malyngamide K	7ug/ml	6ug/ml			Wu *et al*., (1997)
malyngamide L	15ug/ml	8ug/ml			Wu *et al*., (1997)
malyngamide O			2ug/ml		Gallimore *et al*., 2000)
malyngamide R		18ppm			Milligan *et al*., 2000)
microcolin A				TC_{50}=22.6nM	Koehn *et al*., (1992)
microcolin B				TC_{50}=191.0nM	Koehn *et al*., (1992)
obyanamide			0.58 & 3.14ug/ml		Williams *et al*., (2002)
pitiamide A				fish antifeedant	Nagle *et al*., (1997)
pitioeotolide A			weak		Luesch *et al*., 2001)
pitioeotolide B			weak		Luesch *et al*., 2001)
somocystinamide A			1.4g/ml		Nogle and Gerwick, (2002)
tanoikolide		3.6ug/ml		molluscocidal LD_{50} = 50.9ug/ml	Singh *et al*., (1999)
yanucamide A		5ppm			Sitachitta *et al*., 2000)
yanucamide B		5ppm			Sitachitta *et al*., (2000)

LPS has been purported to have an effect on toxin metabolising enzymes. The cytotoxicity of some compounds may be due to their metabolism in the skin, notably by keratinocytes known to express xenobiotic metabolising enzymes (phase I and II)

(Bickers *et al.*, 1982; Cotovio *et al.*, 1997; Gelardi *et al.*, 2001). Crude extracts may contain factors that lead to a differential effect on skin metabolising enzymes. An up-regulation of enzymes producing a more toxic by-product(s) of the original toxins applied to skin, or a down regulation of enzymes catalysing a detoxification pathway may lead to increased activity of toxin above what was seen for purified samples. Alternatively, not the toxins themselves but other factors affecting oedema may be up- or down-regulated.

LPS may have differed on *Lyngbya* containing LA, DAT may have interfered with the effect of LPS, or possibly another mechanism was working as purified LA and DAT responses were similar. It should be noted that some crude extracts containing neither LA or DAT saw increased oedema, suggesting LPS may be acting alone, or alternatively, another irritant was present.

3.5.6 Hypothesis for Differences In Oedema

One possible reason for the differences found between purified toxin and crude extracts is that other chemicals are present that may cause or inhibit inflammation or other toxicological processes. It must be remembered that *L. majuscula* has had over 120 chemicals extracted and characterised from it, and many of these possess biological and toxicological activities. Production of crude extracts with acetone in this experiment would have selectively removed some substances, but may have made others more bioavailable. Some other examples with other cyanobacteria are given below.

Tumour promoters such as teleocidin B have been found to stimulate superoxide anion radical ($O_2^{-\cdot}$) by human polymorphonuclear leukocytes (Goldstein *et al.*, 1981). Similarly teleocidin, DAT and AT stimulated H_2O_2 production by human

monocytes (Keisari *et al.*, 1985). Cyanobacterial LPS has been found to inhibit the detoxification enzyme glutathione S-transferase (GST) (soluble form), where no inhibition of response was witnessed with either *Salmonella* or *E. coli* LPS (Best *et al.*, 2000; Best *et al.*, 2002).

Gaveriaux *et al.* (1988) found that protein kinase C activators of the teleocidin family decrease the IgE-binding capacity of rat basophilic cells. This could be interpreted that the presence of LA may decrease the immunological response of an organism to challenge with the down regulation of IgE receptor expression.

Release of choline by teleocidins (including LA) or DAT may have an effect on membrane permeability. Released choline may be taken up by nicotinic acetylcholine receptors, which appears to be the rate limiting step, converted to acetylcholine and released causing a change in ion channel permeability leading to change in membrane permeability and oedema.

Jungmann and Benndorf (1994) found no correlation between microcystin concentration of microcystis samples and the ability of these to cause toxicity in *Daphnia pulicaria.* Jungmann (1992) had previously shown that toxicity of water extracts of *Microcystis* to *Daphnia* were high in microcystin-free fractions. The presence of a "*Daphnia*-toxic compounds" was therefore proposed, which were present at comparable levels in natural and laboratory cultures (Jungmann and Benndorf, 1994).

Garbacki *et al.* (2000) examined the effect of capsular polysaccharides from several species of cyanobacteria and found inhibited croton oil-induced oedema in mice ear skin. Dermal application of hot water extracts of *Phoridium* spp. collected in Papua New Guinea, Arabia Gulf and Corsica reduced ear swelling by between 9.2-56.2 percent. In contrast, another species of *Phoridium* and a terrestrial *Nostoc*

microscopicum increased swelling by 29.1 and 7.2 percent respectively. In fact, within species differences are known, with a 79.4 percent difference in ability to alter inflammation exhibited by the species *Phoridium* compared with *Ectocarpi*, both collected in Corsica and yielding similar concentrations of crude capsular polysaccharide.

C-phycocyanin, a protein-bound pigment found in many cyanobacteria (at doses 50, 100 or 200 mg/kg p.o.), reduced inflammation in both the carrageenan- and glucose oxidase-induced mouse paw oedema test, and as well as TPA- and arachidonic acid-induced mouse ear oedema test by between 4.43 and 66.6 percent (Romay *et al.*, 1998a; Romay *et al.*, 1998b).

Other chemicals present in cyanobacteria also have been found to be anti-inflammatory in nature. These include tolypodiol a diterpenoid isolated from *Tolypothrix nodosa* (Princep *et al.*, 1996) and ambigol A and B isolated from *Fischerella ambigua* (Falch *et al.*, 1995).

Many biologically derived chemicals have been found to reduce inflammation or the activity of tumour promoters. Chung *et al.* (2001) found topical application of the phenolic phytochemical (ginger derived) substance [6]-paradol and analogues has the ability to significantly reduce both the tumour promoting activities on the dorsal skin of mice and oedema in mice ear by TPA. Koyuncu *et al.* (1999) found that the flavanoid hesperidin inhibited inflammation of mouse ears by phorbol ester tumour promoter TPA. Topical application of hesperidin reduced the number of epidermal layers (100%), epidermal thickness (93.3%) and leukocyte infiltration (73.9%), as inhibiting increase in ear weight (45%). Organic extracts of the Indian perennial herb *Tephrosia purpurea* reduced the croton oil (phorbol ester-induced tumour promotion) response in mouse skin (Saleem *et al.*, 2001).

Pietsch *et al.* (2001) postulate that the up-regulation of guajacol peroxidase (POD) might be due to detoxification reactions in the cells other than found for the detoxification of pure toxins (microcystin) catalysed by GST. Elevation in POD can be correlated with the production of reactive oxygen species such as H_2O_2 and O_2 as associated with damage to the cells membranes, proteins and DNA (DiGiulio *et al.*, 1989). This group further postulate that compounds other than the toxins (i.e. measured microcystins) from the crude extracts may liberate or act as reactive oxygen species, causing the up-regulation of POD (Pietsch *et al.*, 2001).

3.5.7 Time Course of Inflammatory Response

Timing of inflammation was measured to determine if differences existed between LA and DAT, and therefore possibly indicating differential mechanisms of inflammation. No difference existed, suggesting that LA and DAT may be acting via the same mechanism to cause ear swelling. Differences have been found to exist in the time that maximal inflammatory responses to an irritant occur. Patrick *et al.* (1985) found maximal response to phenol occurred at 1 hour, SDS at 2 hours and croton oil at 3 hours. Similarly, using temperature of mouse ears as a measure of blood flow, Patrick *et al.*, (1985) were able to show differential time courses of inflammatory response to croton oil, ethyl phenylpropiolate and methyl salicylate.

Differences were found however in the time that maximal inflammation was reached for crude extracts and purified LA and DAT. Differences in time course of the reactions could be due to differences in rates of absorption, differences in inflammatory mechanisms, differences in mechanisms of repair or a combination of these, or other factors (Patrick *et al.*, 1985). Changes in rates of absorption of LA or DAT due to vehicle would not occur as DMSO was used for all samples, both crude

extracts and purified. However differences in rates of absorption of other chemicals present in crude extract cannot be discounted. As LA and DAT are both strong irritants and used in similar doses, it can be presumed that similar mechanisms are at work. So it may be hypothesized that either a repair mechanism was hindered or other toxins or modulating factors were present in the case of crude extracts of *Lyngbya.*

In the case of microcystin, rate of uptake seemed to be an issue in the differences between crude extracts and purified samples (Fastner *et al.*, 1995; Oberemm *et al.*, 1997). Another mechanism is probably responsible in the case of toxins from *Lyngbya* as uptake and onset of reactions are rapid for these toxins with increases in ear thickness occurring in 15 minutes. Interestingly this fast response is not recorded in humans that are exposed to *L. majuscula*, with skin patch testing in humans revealed pruritus began 4-7.5 hours after application of cultures of pure *Lyngbya majuscula* (Grauer and Arnold, 1961). Contractions by LA on rabbit aorta took longer that other tumour promoters, requiring 3 hours to become maximal (Robinson *et al.*, 1991). Ohuchi *et al.* (1987) found that the vascular permeability as measured by the leakage of labeled albumin *in vivo* occurred within 30 minutes of application of tumor promoters including AT, matching the effects seen in the mouse ear in these experiments.

3.5.8 Histopathology

Irritation to the mouse ear in these experiments appears to occur in several stages. Initially there is an increase in ear thickness (first measured at 15 minutes). Ear thickness again increases at about 90 minutes (Figure 3.9). Steele and Wilhelm (1966; 1970) examined the time sequence of irritant inflammation and found a

triphasic system. During the first stage increased blood flow and increased permeability responses began to develop simultaneously. Inflammatory cells infiltrated the area within 5 minutes. These effects then altered with the initial erythematous response fading rapidly while vascular leakage returned slowly to normal by 90 minutes. The second phase of inflammatory response then began with leukocytosis, increased blood flow and the return of erythema, and a return to normal vascular permeability and occurred between 2 and 10 hours. The third stage consisted of a period of increased vascular permeability occurring sometime between 10 and 36 hours (Steele and Wilhelm, 1966, 1970). The period of time covered in these experiments was from 15 minutes to 150 minutes. During this time some of the features mentioned in the description of inflammation by Steele and Wilhelm (1966, 1970) after chemical injury do occur, whilst other do not.

Initially there was a thickening of the mouse ear, seen at the first measurement at 15 minutes. This initial inflammatory response, similar to Steele and Wilhelm (1966) then appeared to subside with little inflammation occurring at 90 minutes.

Unlike the Steele and Wilhelm (1966, 1970) inflammation model, ear thickness again increased at 90 minutes. In their model vascular permeability remained low until 10 hours after application of chemical irritant. But similar to their model was the influx of neutrophils during this second stage. It should be noted that Steele and Wilhelm (1966) examined the effects of organic solvents on guinea pigs as well as using different methodologies to measure inflammation, which may explain some differences (Patrick *et al.*, 1985). Pearlman *et al.* (1999) noted that in contact hypersensitivity neutrophils predominated for the first 12 hours, with only the occasional eosinophil, and that this ratio was reversed at later times and lasted up to 7 days.

Different mechanisms of skin inflammation have been documented. Young and De Young (1989) postulated that tumour promoter TPA induced inflammation via arachidonic metabolites in ear tissue. This is a probable avenue for the action of DAT and LA as the both may be working via similar receptors. Other mechanisms of inflammation include the neurogenic inflammation caused by capsicaicin where neuropeptides from primary afferent neurones are released (Holzer, 1988). Inoue *et al.* (1997) examined another pathway involving the tackykinin NK_1 receptor in response to application of mustard oil. These models of contact irritant dermatitis differ again from dermal contact hypersensitivity reactions.

LA and DAT produced similar histopathological outcomes at similar degrees of damage in similar timeframes. Both LA, DAT and LA+DAT had some oedema at 15 minutes while at 150 minutes there was more extensive oedema as well as an influx of neutrophils. Patrick *et al.* (1985) noted that different irritant chemicals differed in time course of development of oedema, cellur infiltrates and vessel dilation. These irritants included phenol, benzalkonium chloride, methyl salicylate, croton oil and ethyl phenylpropiolate.

Croton oil, containing phorbol ester tumour promoters, has been found to become oedematous within 1 hour (Patrick *et al.*, 1985). However, there was no increase in the number of visible blood vessels. Only after 6 hours were increased number of blood vessels visible, oedema was moderate to severe and a diffuse and vascular cellular infiltrate had developed. Vessels at the ear margin had not dilated. The actions of LA and DAT and LA+DAT appear to be faster, although the actual concentrations of active components in croton oil and hence dose is unknown. DMSO was also used as a vehicle, which may have led to increased penetration of

toxin and hence earlier inflammation, although this has been disputed by some (Patrick *et al.*, 1985).

LA has been found to have several effects on mice, including increasing the permeability of blood capillaries and stimulating the hyper-secretion of "strongly sticky juice" from gastric glands (Ito *et al.*, 2002) after gavages at 600 and 1000 μg/kg. It has also been found to have suppressive effects on mitosis (Ito *et al.*, 2002).

The mechanisms by which chemicals applied to the skin induce irritant inflammation have not been described in detail (Patrick *et al.*, 1985). The tumour promoter phorbol esters TPA, phorbol dibutyrate (PDBU), teleocidin and AT, are all known protein kinase C activators. *In vitro*, these caused a decrease in transepithelial gradients and resistance, which were paralleled by a rise in the transepithelial (paracellular) flux of D-mannitol between the cells (through the tight junctions). This evidence supported the case for protein-kinase-C-mediated control of tight junctional permeability (Mullin *et al.*, 1990). Vascular permeability is thereby increased, leading to an influx of fluid into the lamina propria.

In our experiments, no histopathological difference was detected between LA and DAT, although some authors have previously found differences. Differential effects of AT and LA in mice gastrointestinal histopathology have been noted. LA was found to cause a filling up of the space between the lamina propria and epithelial cells in the intestine with exudate (Ito *et al.*, 2002). Ito and Nagai (1998; 2000) dosed mice with aplysiatoxin (AT) and saw the dilation of lymphatic vessels preceding blood infusion into the lamina propria from the submucosa, again in the small intestine of mice. In human skin, a biopsy revealed superficial desquamation and oedema of the epidermis with some round cell infiltrate in the immediate subepidermal areas. A few

scattered eosinophils were noticed, and some microvesiculation (Grauer and Arnold, 1961). These are similar to what was observed in the mouse ear in this research.

3.5.9 Recovery of Mouse Ears

In general, it took approximately ten days for the mouse ear thickness to return to normal, irrespective of the dose. Patrick *et al.* (1985) also found a lack of correlation between intensity of the acute ear thickness response and duration of increases in ear-thickness. These results suggest that either there are parameters of the inflammatory response not measured by ear thickness, or the response of permanent ear thickness is related to a non-inflammatory mechanism. An example of ear thickness not relating to the inflammatory response with phenol binding to protein in mouse skin and hence thickness (Roe, 1957, cited in Patrick *et al.*, 1985).

Previous work has show differences in recovery times for different irritants. Patrick *et al.* (1985) found that ears dosed with croton oil, ethyl phenylpropiolate or methyl salicylate retuned to normal but some ears on which phenol or benzalkonium chloride was applied remained thickened for more than 4 weeks.

LA may have a suppressive effect on mitosis. Mice exposed to LA via i.p. or p.o. had a marked increase in the number of mitotic cells in the liver after 4 days, despite no significant injuries prior to this. Ito *et al.* (2002) postulated that this increase in the number of mitotic cells possibly reflects the recovery of mice from the suppressive effects of LA. Hence LA may inhibit recovery from cellular damage.

3.5.10 Conclusions

- DAT and LA have similar irritant effects.
- No synergistic relationship exists between these two toxins.
- The toxicity of crude extracts of *Lyngbya* containing LA are not fully explained by the measured LA content.
- No enhancement of toxicity with DAT-containing *Lyngbya* extracts was found.
- *Lyngbya* containing no LA or DAT exerted an irritant effect.
- Other toxins or modulating factors appear to be present.
- LA and DAT have similar histopathological actions
- Time courses of LA and DAT pathology are similar.
- Crude extracts have a different time course of irritation.

4. Environmental Toxicology of *Lyngbya*

4.1 Introduction – Environmental Toxicology

The presence of a *L. majuscula* bloom does not automatically mean an increase in risk to human or ecological health. Blooms in the past have been characterised by their transient nature, lasting only a matter of weeks to months (Watkinson, 2000). While this may pose some direct threat to ecological health in the way of shading of sea grasses and impact on organisms utilising sea grass as a food source, the toxicity of *Lyngbya* has been viewed as the major threat to human health as well as ecological health. Human health is affected by exposure to the toxins in sufficient quantities, not the presence of *L. majuscula*. Similarly the toxins in *L. majuscula* have been reported to be antifeedants (Pennings *et al.*, 1996; Thacker *et al.*, 1997; Nagle and Paul, 1999), so if no toxin is present, grazers could potentially reduce its ability to bloom, or lead to a quicker reduction once it has bloomed.

In order to assess these risks, certain parameters must be measured. These include what toxins are present, consistency of levels, geographic distribution pattern, spatial pattern in relation to time, season, bloom size, or other environmental parameters. By better understanding bloom dynamics and factors influencing toxicity, better predictions can be made on the potential harmful effects during a bloom. Anderson (1996) recommends measurement of inorganic nutrients and their sources, as well as other phytoplankton growth factors, in planning and operating a monitoring program.

Two theories exist of why primary producers differentially increase levels of toxic secondary metabolites (Waterman and Mole, 1994). Stress appears to be a major contributing factor. Levels of potentially toxic secondary metabolites have been found to increase once an organism is under stress (in both vascular plants and

cyanobacteria). This may include high or low levels of nutrients, micronutrients, extremes of temperature, intensity and length of light exposure or shading, and degree of salinity. Other factors include degree of grazing and physical disturbance.

Toxin levels may also be affected by the period of growth. If an organism is growing at a rapid rate, it has energy that it can spend on producing secondary metabolites for its protection from grazers. These two factors probably also interact with one another. For example, once the bloom has reached its peak, nutrients may become limited or at the peak grazers have had time to increase in numbers to consume the bloom.

While some groups have cultured *L. majuscula* (Armstrong *et al.*, 1991; Rossi *et al.*, 1997; Burja *et al.*, 2002), the ability of this technique to assess effects of environmental parameters was thought to be limited with culturing techniques in their present form. The main objective was to determine effects in the field of various parameters on the growth and toxin levels of *L. majuscula.* In an attempt to better understand the toxin levels of *L. majuscula*, samples were analysed from different times and locations to establish if any correlation could be made between toxin levels and environmental, meteorological and geographic parameters.

4.2 Methods - Environmental Toxicology

4.2.1 Introduction

This chapter deals with the work performed to extract, purify and characterise toxins of the species *L. majuscula.* The initial extractions focused on two toxins of this species, LA and DAT, as standards of these were available (thanks to Prof. R. E. Moore, University of Hawaii). Areas sampled were in SE Queensland, primarily the North Deception Bay and Eastern Moreton Bay *L. majuscula* blooms of 2000, 2001 and 2002. Anecdotal evidence had suggested variable toxin levels and distributions (Mynderse *et al.*, 1977; Cardellina *et al.*, 1979; Aimi *et al.*, 1990), and this was investigated by selection of *Lyngbya* samples from differing locations, times and, states of growth.

4.2.2 Chemicals

Solvents used in extractions included methanol, dichloromethane, hexanes (nanograde - 95% n-hexane) (Mallinckrodt, Paris, USA), propan-2-ol, chloroform, (BDH, Kilsyth, Australia), acetonitrile, acetone (EM Science, Gibbstown, USA) and dimethylsulphoxide (AMRESCO, Solon, USA). All chemicals used were of analytical grade or higher.

4.2.3 Sample Collection

Samples were collected from a variety of sites around SE Queensland. These included Pumicestone Channel (-26°56'S: 153°04'E), Deception Bay (-27°08'S: 153°05' E), Amity (-27°26'S: 153°22'E) and Myora (-27°28'S: 153°25'E). Sampling involved collection and identification of *L. majuscula.* Cyanobacteria were stored in seawater until returned to the laboratory and frozen and stored (0°C) within eight

hours. To assess the variation of *L. majuscula* within a bloom twenty grab samples were taken within a circle with a two-meter radius.

4.2.4 Chemical Extraction

Frozen samples of *L. majuscula* (~20g) were lyophilised with a freeze-drier (FD12 and FDA/3RHC Dynavac, Hingham, USA) until dry and ground using a coffee grinder Phillips, Australia). Samples were extracted with 50 ml of acetone overnight after 20 minutes in a sonicating water bath (Branson Ultrasonics, Danbury, USA). The extract was then filtered through GF/C glass fibre filter paper (Whatman, Maidstone, UK) under vacuum into a Büchner flask to remove particulate matter. Solvent was then removed by rotary evaporation (Büchi, Switzerland) at less than 40°C. Residue was resuspended in 6 ml of 50% aqueous methanol and filtered using 0.45 μm disk filter (Millipore, USA).

4.2.5 Air Sampling

A high volume air sampler at Deception Bay was used to examine if *L. majuscula* toxins LA and DAT were present in air in the area near blooms. Samplers were run with a TSP size selector at flow rate of 1.13 m^3 / minute. Filters were examined after running the sampler for 24 hours. Acetone extraction of filters was undertaken followed by examination as described above using LC-MS. Filters were examined using a bifocal microscope in an attempt to determine the presence of filaments of *L. majuscula*.

4.2.6 Environmental Data

Data on water quality parameters was obtained from the Environmental

Protection Agency, Queensland Government for sites 1118 (North Deception Bay) and site 507 (Eastern Moreton Bay). Weather data was procured from the Bureau of Meteorology, Commonwealth of Australia and was from the Brisbane Airport weather station (-27° 24' S: 153°06' E).

4.2.7 Analytical

Triple quadrupole LC/MS/MS mass spectrometry (Perkin-Elmer Sciex Instruments) was used for this analysis. The LC column was an Altima C18 (Alltech) 150 mm x 4.6 mm. Flow rate was 0.8 ml/min. Isocratic elution was used and the mobile phase consisted of 65% solvent A (60% CH_3CN / 0.1% formic acid), 35% solvent B (95% CH_3CN / 0.1% formic acid). All peaks eluted over 17 minutes. Injection volume was 10 µl. Eluted product was split 1:4 to the MS. Polarity was positive. LA was measured at molecular ion ($M+H^+$) 438.3 and the fragment at 410. DAT was measured with ions ($M+Na^+$) 615.3 and fragment 543.3.

The method for examination of saxitoxins in cyanobacteria was derived from the method of Lawrence *et al.* (1995) used to examine shellfish. Freeze dried samples of *L. majuscula* were sonicated with a Branson 450 watt ultrasonic probe and filtered using a PVDF 0.45 µm (Millipore, USA). Prechromatographic oxidation was carried out by firstly adjusting the pH by adding 250 µl of 1 N NaOH to 100 µl of sample and then adding 25 µl of hydrogen peroxide and allowing to react for 2 minutes at room temperature. Reaction was stopped by addition of 25 µl of glacial acetic acid.

Saxitoxin oxidation by-products were chromatographed using an Alltima C18 column (150 x 4.6 mm, Alltech, Australia), with a gradient of acetonitrile in 0.1 M ammonium formate, adjusted to pH 6 with acetic acid (flow 1.0 mL/min., 1% acetonitrile to 8% acetonitrile in 20 minutes). Saxitoxins were quantified using a

Shimadzu LC-10ADVp HPLC system with a Shimadzu RF-10AXL fluorescence detector set at an excitation wavelength of 330 nm and an emission wavelength of 390 nm. There are over 12 known toxins related to saxitoxin, of which saxitoxin is the most toxic. Quantitation was performed by comparing the peaks identified in the sample to the area of peaks of a saxitoxin standard of known concentration (NRC, Canada), and were reported as total saxitoxins (mg/L). The detection limit for this method was 2 µg/L for individual saxitoxins, and a sample of *L. wollei*, a species known to contain saxitoxins, was used as a positive control.

4.3 Results – Environmental Toxicology

4.3.1 Spatial Differences

Distinct patterns of distribution of LA and DAT were found across South East Queensland. Samples collected between April 1999 and December 2002 displayed similar toxin patterns in samples from the western side of Moreton Bay, including Waterloo Bay, N. Deception Bay, Godwin Beach, Pebble Beach and Bongaree containing variable levels of DAT (BDL-43.0 mg/kg) but LA was BDL (below detectable limit). This pattern was reversed on the Eastern Moreton Bay including Amity Banks, and Moreton Banks, with LA being present (BDL-131.9 mg/kg) while DAT was BDL (Table 5.1). Defying this trend were four samples collected in January and April 2002 from East Moreton Banks, which contained DAT at levels between BDL-5.37 mg/kg. Levels of toxins varied greatly from BDL-131.9 mg/kg in the case of LA and BDL-43.0 mg/kg for DAT.

4.3.2 Temporal Distribution

Toxin production varied over time. Blooms of *L. majuscula* occurred during the hotter months of the year, although smaller populations could also be found at other times of the year. Toxin levels of both LA and DAT varied from year to year, both in amount and type (Figure 4.2).

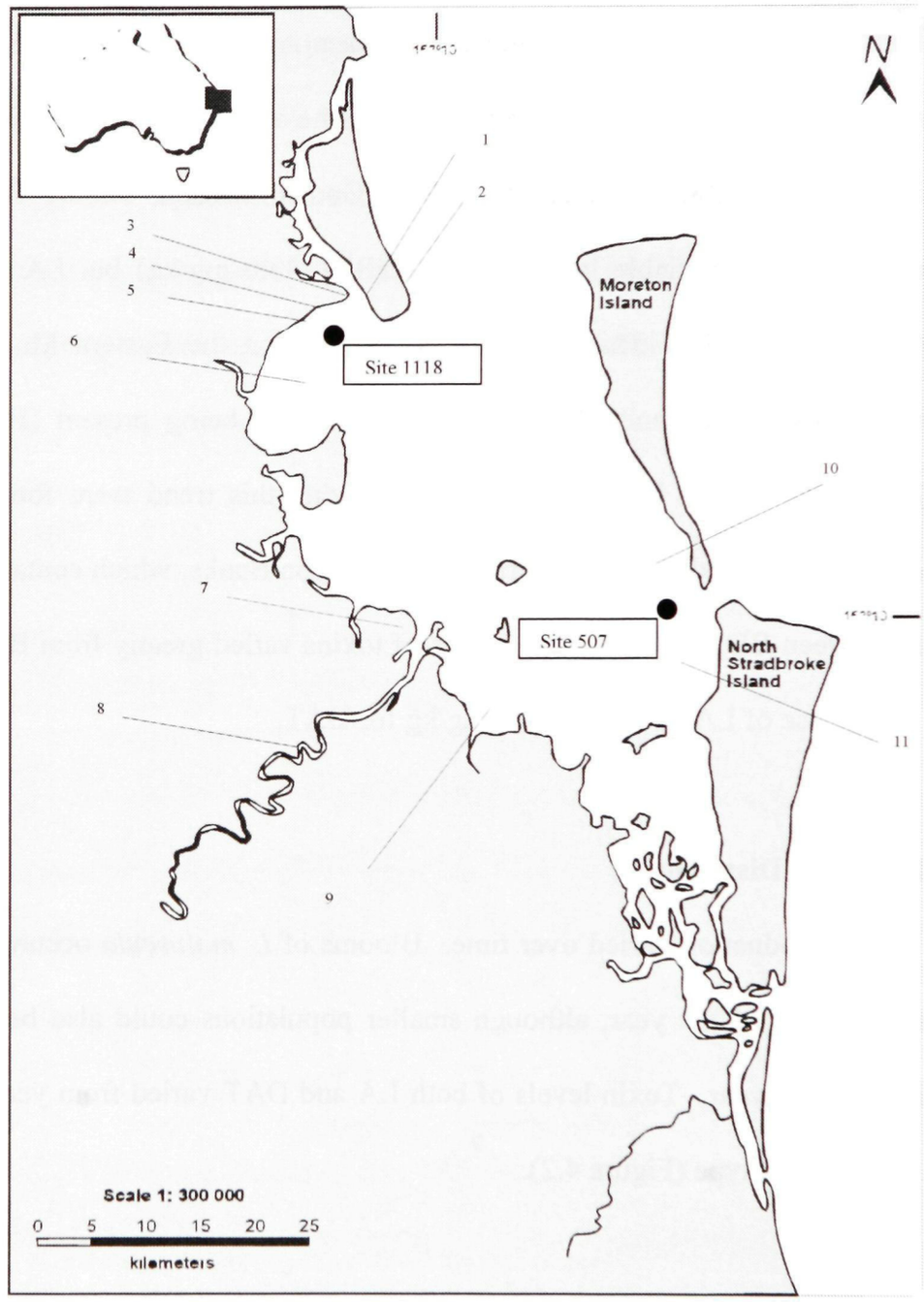


Figure 4.1 Moreton Bay and surrounds with sampling points. 1. Bongaree; 2. Woorim; 3. Sandstone Point; 4. Pebble Beach; 5. Godwin Beach; 6. Deception Bay; 7. Brisbane airport; 8. Brisbane CBD; 9. Waterloo Bay; 10. Moreton Banks; 11. Amity Banks.

Table 4.1 Monthly averages of DAT and LA (mg/kg freeze dried weight) levels found in North Deception Bay (NDB) and Eastern Moreton Bay (EMB) (BDL = below detectable limit).

		n	LA (mg/kg)	st dev	DAT (mg/kg)	st dev
NDB						
February	2000	4	BDL		23.6	0.7
April	2000	6	BDL		0.8	1.0
January	2001	43	BDL		2.3	2.9
February	2001	5	BDL		1	0.9
November	2001	2	BDL		BDL	
EMB						
March	2000	2	6.8	3.7	BDL	
June	2000	1	5.4		BDL	
March	2001	2	51	55.6	BDL	
April	2001	8	38.3	2.0	BDL	
July	2001	1	132.9		BDL	
October	2001	4	12.6	9.0	BDL	
December	2001	2	BDL		BDL	
January	2002	2	0.5	0.7	BDL	
March	2002	4	1.4	0.4	2.8	
April	2002	9	0.56	0.31	BDL	

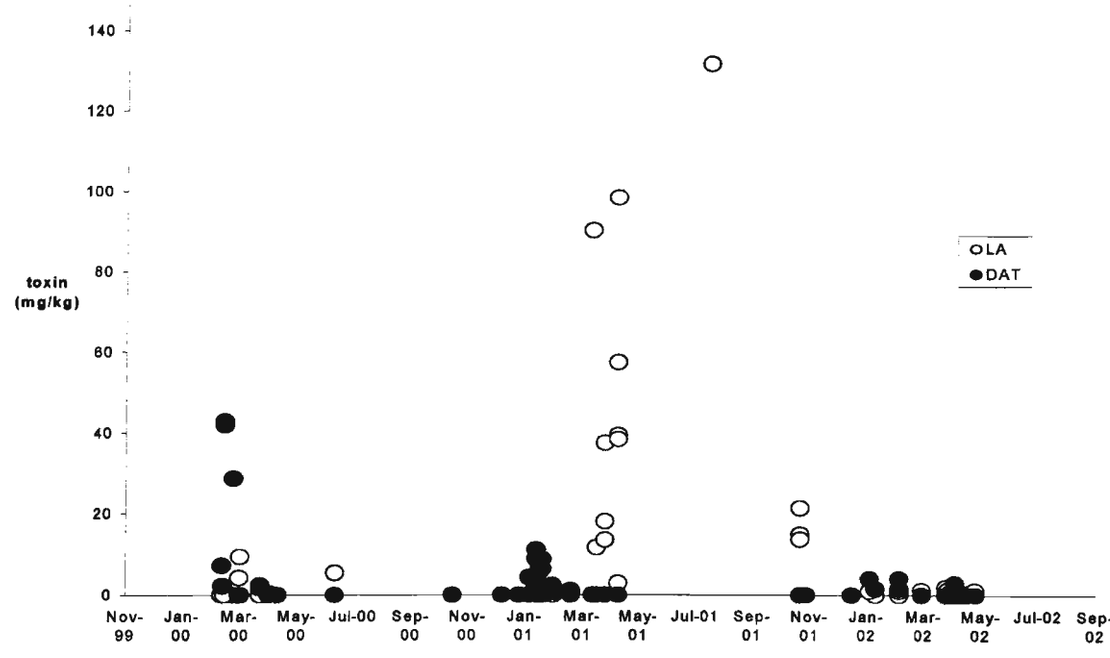


Figure 4.2 Levels of LA and DAT (mg/kg) of samples collected in Moreton Bay from January 2000 to April 2002.

4.3.3 Toxin Levels at a Single Site at a Single Time

Twenty samples were collected within a 2m radius area were collected by snorkelling on 21 January 2001 at Pebble Beach, North Deception Bay. Levels of DAT ranged from 0-11.2 mg/kg with an average of 2.3 mg/kg. LA was below detectable levels. No significant difference in the average DAT concentration was found between the 20 samples collected in the water and 3 samples collected on the beach above high tide mark for that day (t test, $p = 0.88$). Samples collected at the same location four days later also contained only DAT and the average concentration was not significantly different from those collected on the 21/01/02 (Table 4.2).

Table 4.2 Levels of DAT at Pebble Beach, NDB.

average	DAT (mg/kg)	st dev	range (mg/kg)	n
21/01/02	2.32	2.89	0-11.17	20
beach 21/01/01	2.58	0.21	2.34-2.75	3
25/01/02	2.80	3.45	0-8.91	9

4.3.4 Relationship between Toxin Concentration and Growth

Maximum levels of both LA and DAT were found at the height of bloom intensity (Figure 4.3 and 5.4). Toxin levels were low at the beginning of the bloom and reached highest levels at or near maximum bloom intensity. Bloom intensity was determined by examination of Queensland National Parks Service records, which gave account of size of bloom (number of areas effected) and density (percent coverage of sea floor with *L. majuscula* filaments). These were combined to give an estimation of "bloom intensity", rated between zero and ten, with no *L. majuscula* present having a rating of zero, up to 50 % cover at 5 sites being five and up to 100% cover at 10 sites over several banks at Eastern Moreton Bay being ten.

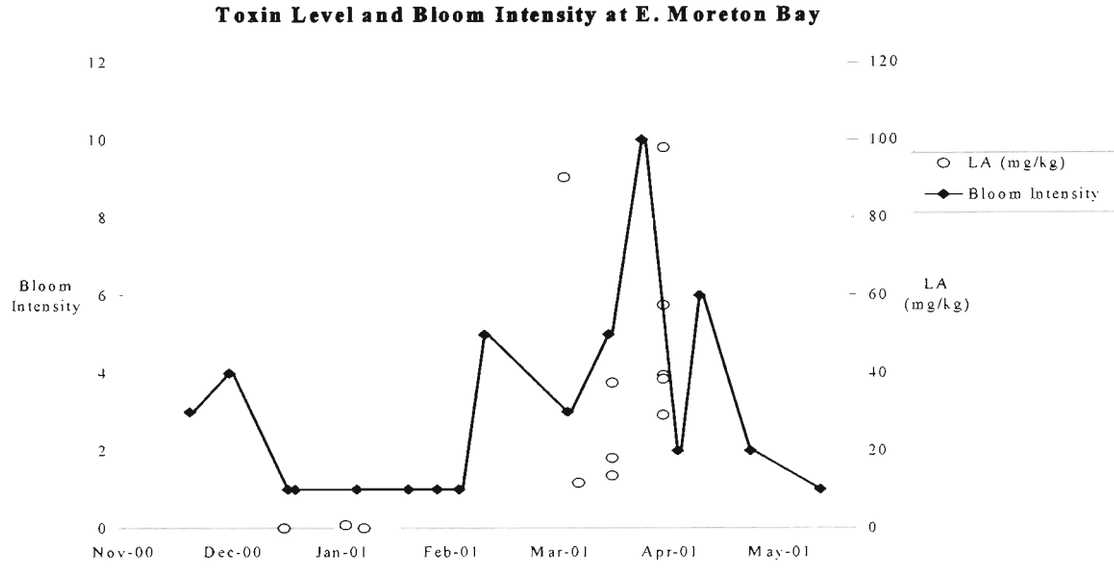


Figure 4.3 Comparison of levels of LA (mg/kg FDW of *L. majuscula*) and bloom intensity in EMB.

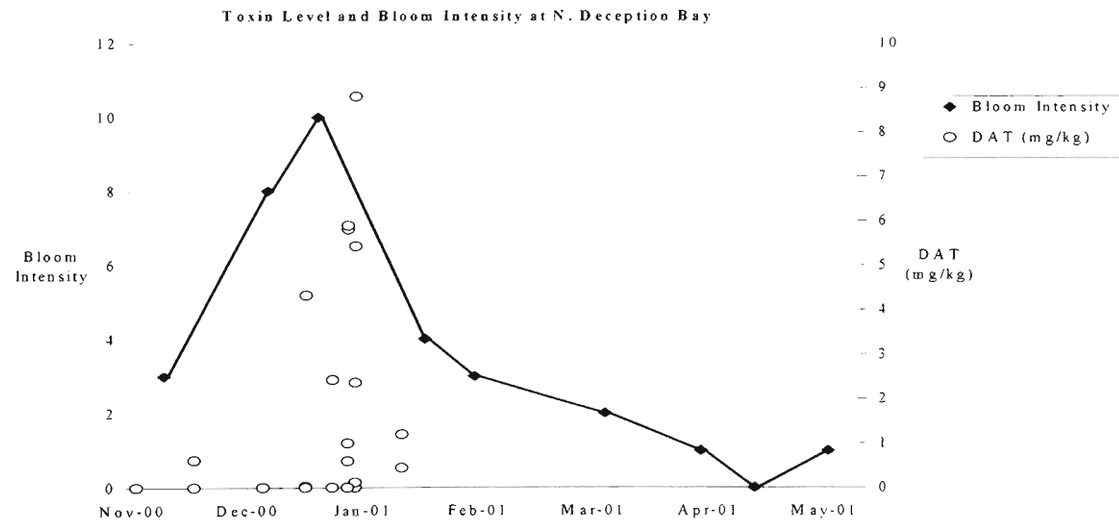


Figure 4.4 Comparison of levels of DAT (mg/kg FDW of *L. majuscula*) and bloom intensity in NDB.

4.3.5 Temperature

Blooms of *L. majuscula* occurred when water temperature increased during the summer months (Figure 4.5). Recorded water temperatures immediately prior to bloom initiation were over 25°C at both NDB and EMB. Water temperature was closely linked to air temperature, both increasing after June.

Table 4.3 Ranges of environmental parameters from March 2000 to July 2001 and levels of parameters at bloom initiation.

environmental parameter	range NDB	range EMB	bloom inititation NDB	bloom inititation EMB
water temperature (°C)	16.4-26.9	17.7-26.2	26.4	26.2
Secchi depth (m)	0.9-4	2.0-5.0	1.8	4
oxygen (dissolved) (mg/L)	6.2-8.1	6.4-8.1	6.8	6.7
oxygen % saturation	93.2-104.2	93.2-108.3	102.3	100.8
N (total) (mg/L)	0.13-0.6	0.1-0.31	0.18	0.13
N (NH4+) (mg/L)	0-0.1	0.002-0.004	0.000	0.002
N (organic) (mg/L)	0.1-0.6	0.1-0.3	0.2	0.1
N (oxidised) (mg/L)	0.0-0.1	0.002-0.008	0.000	0.002
P (total) (mg/L)	0.01-0.044	0.004-0.018	0.03	0.018
P (diss. react.) (mg/L)	0.002-0.019	0.002-0.006	0.01	0.005
pH	8.0-8.4	7.9-8.4	8.2	8.2
conductivity (mS/cm)	35.3-54.9	51.9-54.5	54.4	53.2
salinity (g/L)	22.3-36.4	34.2-36.1	36.1	35.1
chlorophyll-a (µg/L)	0.5-6.6	0.5-1.7	1.7	0.9
pheopigments (µg/L)	0.5-7.9	0.5-0.7	0.5	0.5

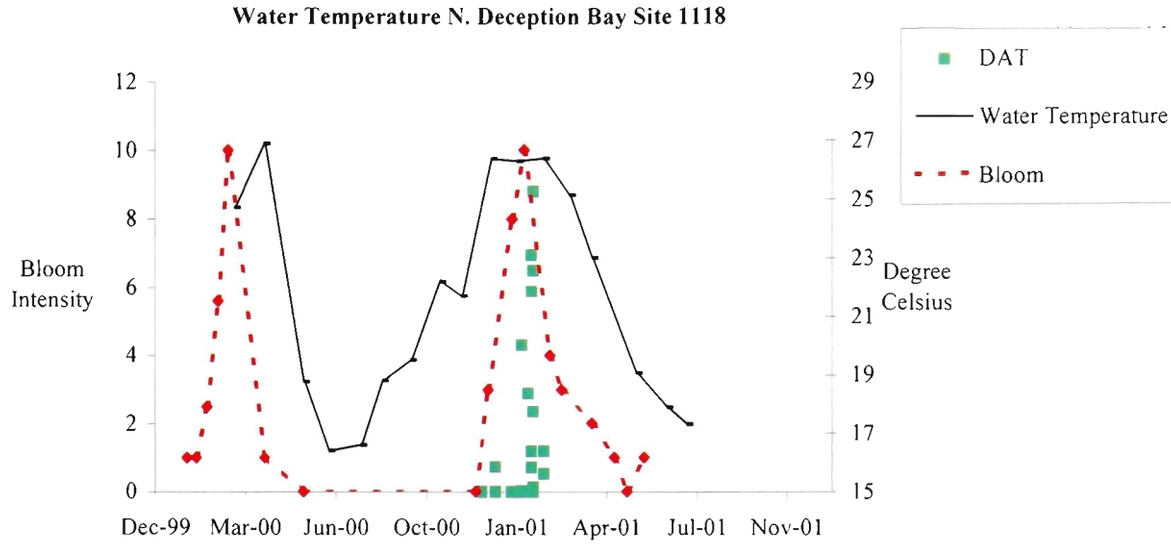


Figure 4.5 Relationship between monthly average water temperature, bloom intensity and toxin levels at NDB.

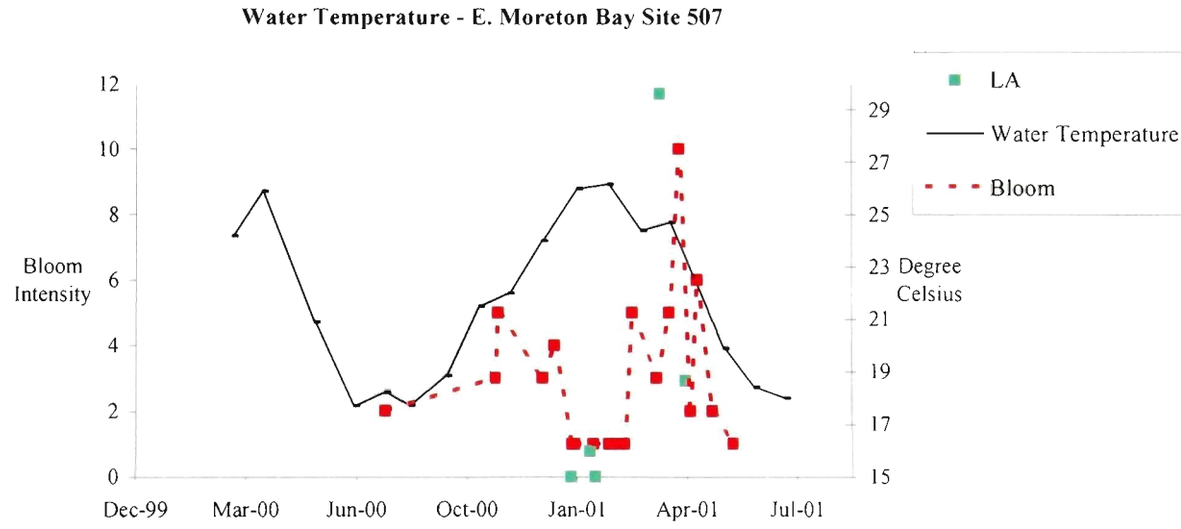


Figure 4.6 Relationship between monthly average water temperature, bloom intensity and toxin levels at EMB.

4.3.6 Precipitation

A two-month period (10 August 2000 to 10 October 2000) of low rainfall existed before the bloom began on EMB. Similarly, one month (24 November 2000 to 24 December 2000) occurred before blooms in NDB. Bloom dynamics are demonstrated in Figure 4.7 and Figure 4.8.

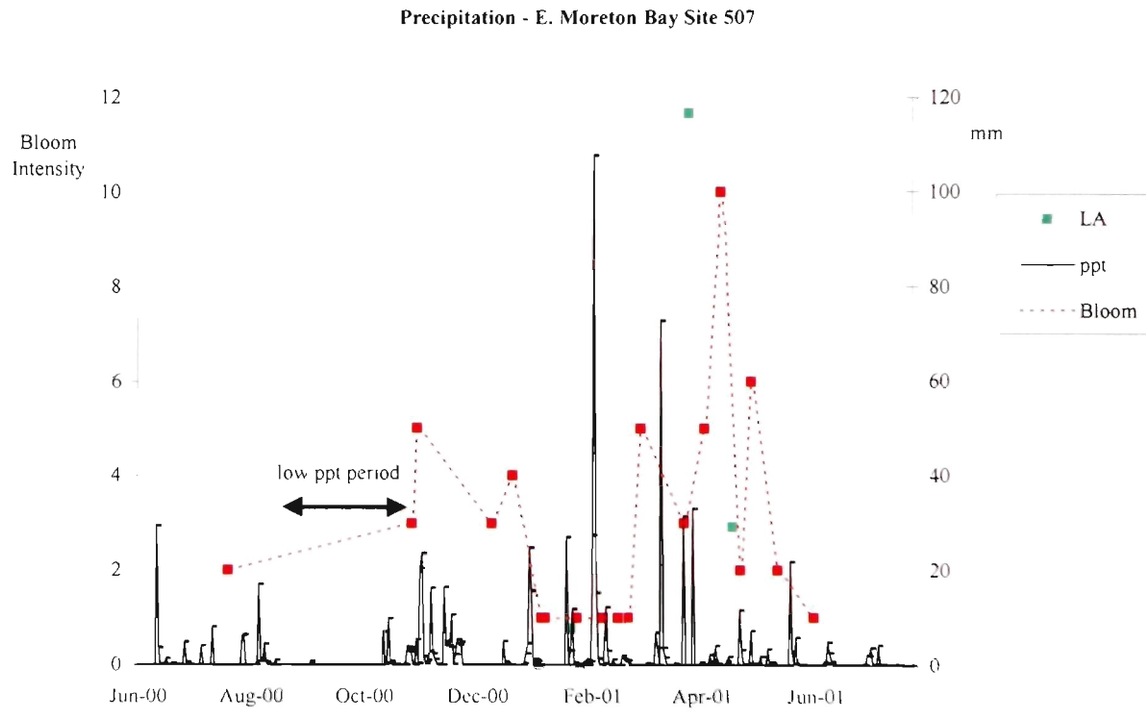


Figure 4.7 Relationship between rainfall and blooms at EMB.

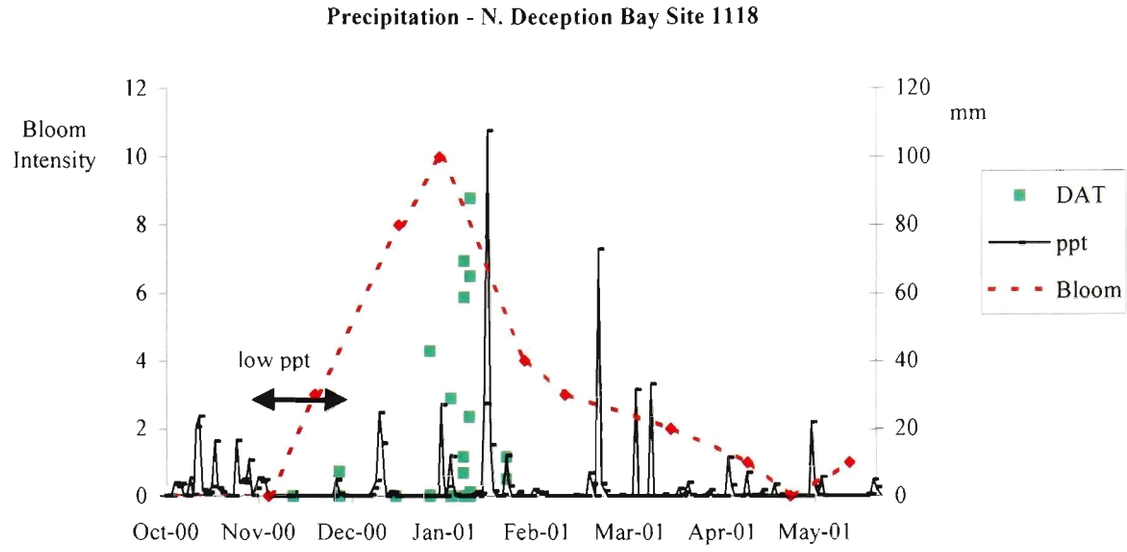


Figure 4.8 Relationship between rainfall and blooms at NDB.

4.3.7 Biotics

During the 2001 bloom episodes chlorophyll-a reached a maximum during the bloom episode, with EMB recording a high of 1.74 µg/L and NDB 6.55 µg/L (Figure 4.9 and 4.10). A similar peak of pheopigments was recorded at NDB (1.87 µg/L) (Figure 4.11) but not EMB (Figure not shown).

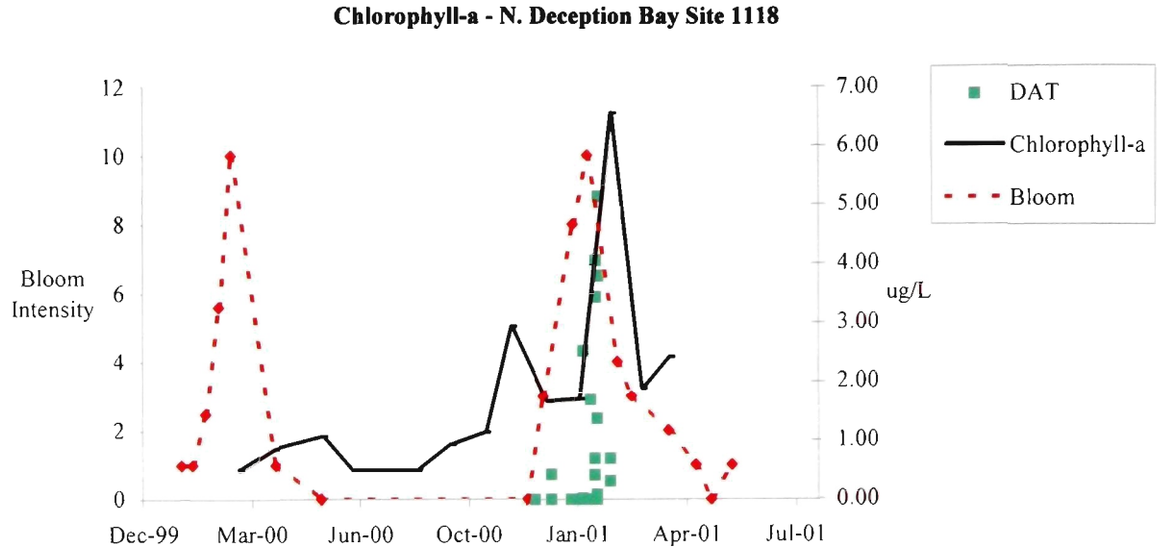


Figure 4.9 Levels of chlorophyll-a with respect to time at NDB.

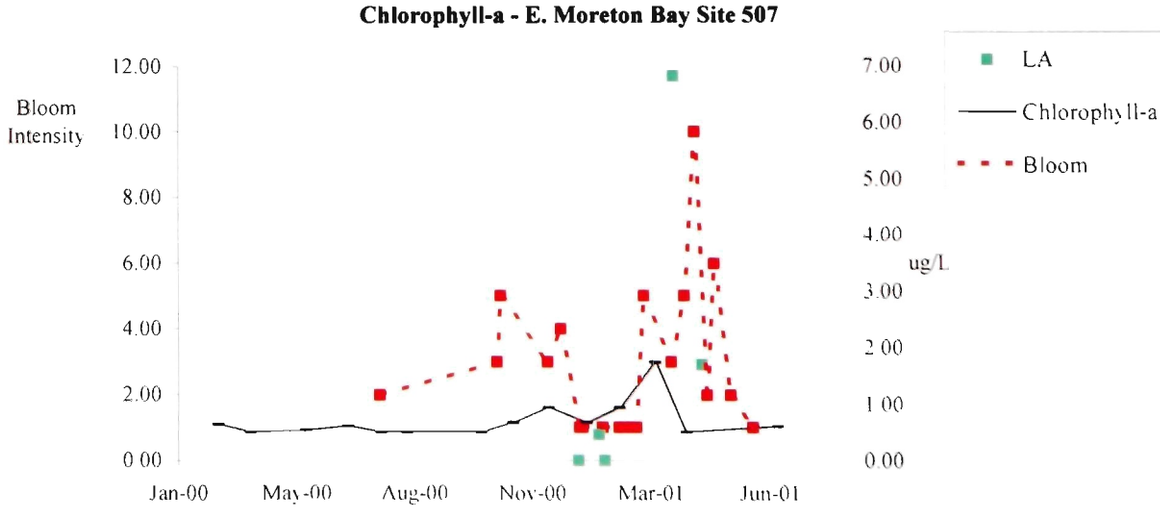


Figure 4.10 Levels of chlorophyll-a with respect to time at EMB.

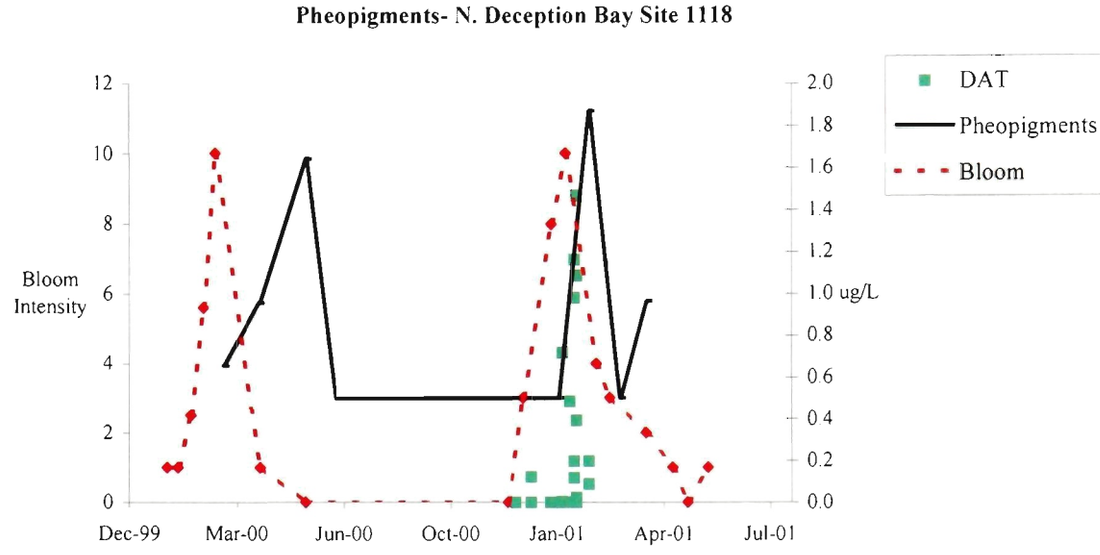


Figure 4.11 Levels of pheopigments with time at NDB.

4.3.8 Other Factors Affecting Bloom Intensity and Toxin Levels

Most of the factors mentioned below did not appear be synchronised with bloom growth. The pH did not vary between NDB and EMB. At NDB pH averaged 8.23±0.01 and at EMB of 8.20±0.1. Salinity (g/L) was effectively stable over the measurement period at approximately 34-36 g/L, except for one measurement at NDB where salinity dipped to 22 g/L for a single reading after a rain event. A similar reduction was not seen at the EMB site.

The Moreton Bay region exhibits typical weather patterns for its subtropical climates. Summers are warm and wet while winters are cooler but dry. During the change over period prevailing winds are predominately from the east. In the year 2001 wind directions came from a South Easterly direction during the "autumn" (monthly average direction of maximum wind gust was ESE), after having been from

the south during winter. This did coincide with bloom formation. Wind speeds increased prior to bloom formation. Monthly average speed of winds increased from 32-38 km/hr to 38-42 km/hr. Wind speeds reduced after March.

Phosphorus was measured as total phosphorus and dissolved reactive phosphorus. During summer there appeared to be an increase in total phosphorus from 0.02 mg/L to 0.04 mg/L. No such movement was noted at the EMB site, where levels varied from 0.004-0.018 mg/L for total P. P was present at the higher levels at bloom initiation for NDB and EMB. During the bloom episode at NDB nitrogen (ammonium) peaked at 0.01 mg/L for one measurement before returning to 0.002 mg/l. No such peak was witnessed at EMB. Oxidised nitrogen (NO_2 and NO_3) did not change from 0.02 mg/L during the bloom episodes on both NDB and EMB.

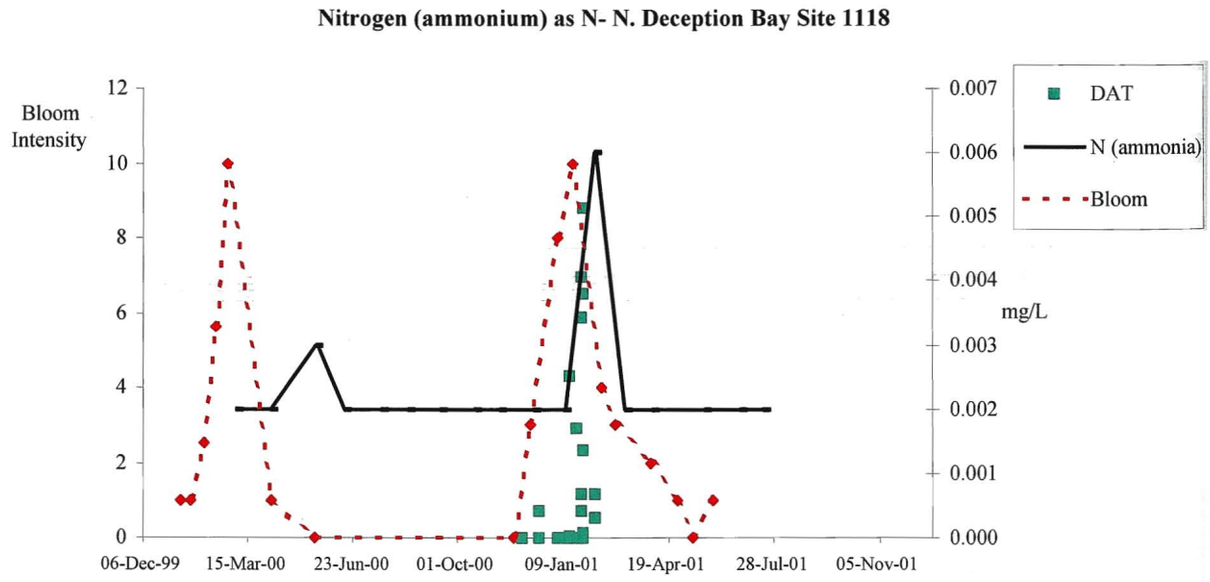


Figure 4.12 Changes in ammonia during a bloom at NDB and its relation to toxin level.

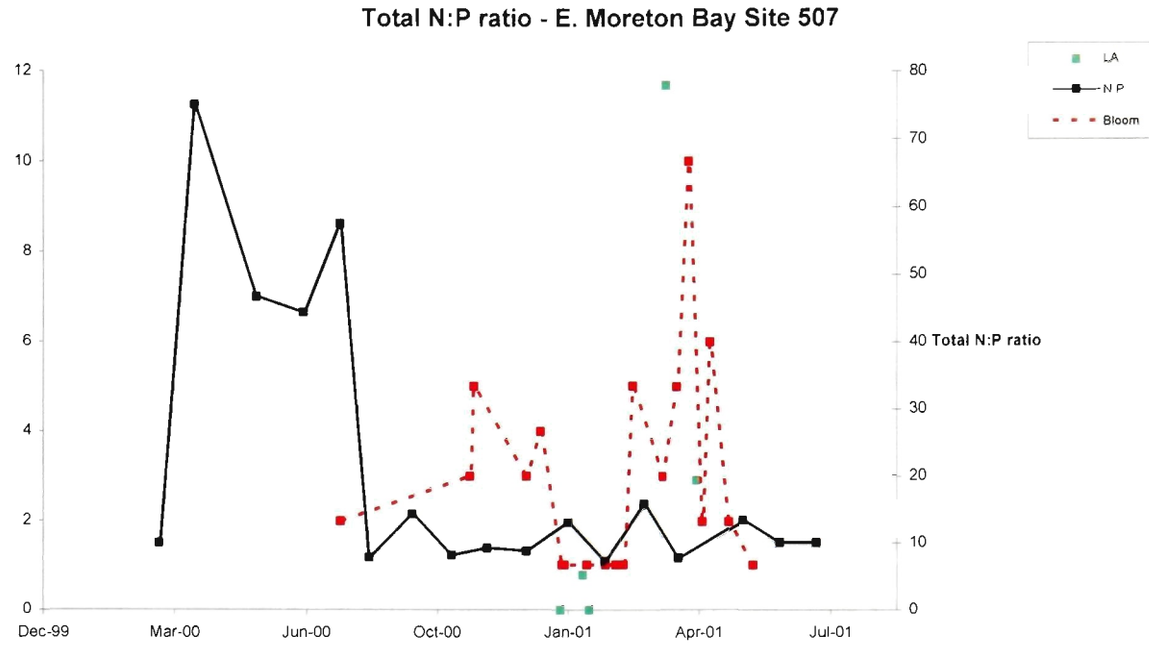


Figure 4.13 Relationship between bloom, toxin production and total N:P ratio at EMB.

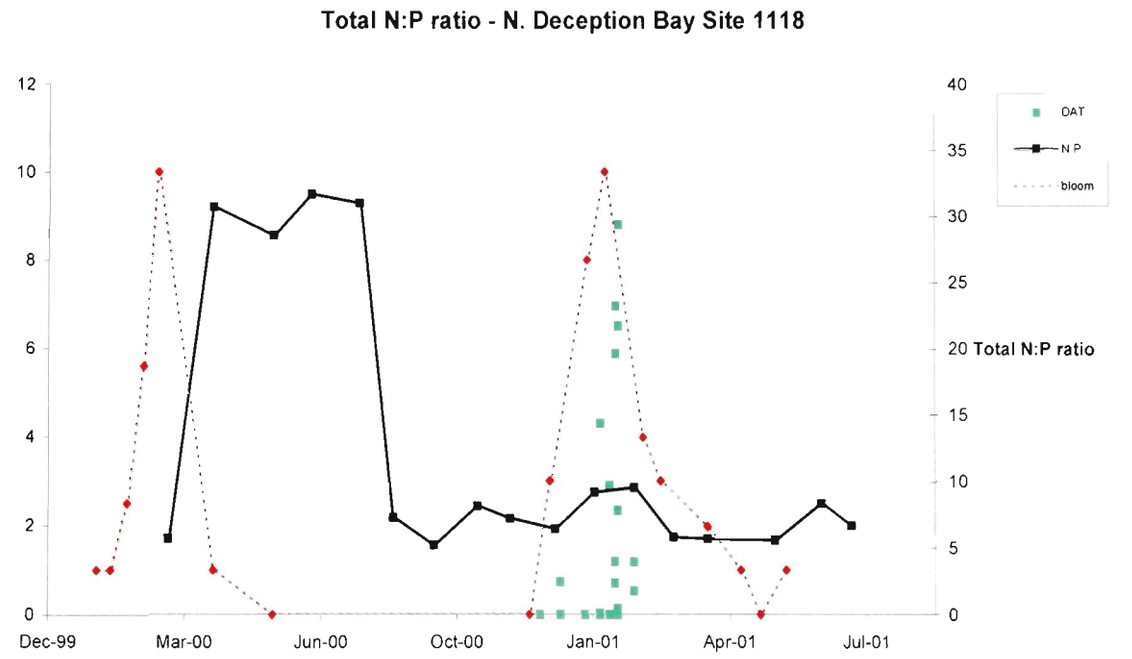


Figure 4.14 Relationship between bloom, toxin production and total N:P ratio at NDB.

The ratio of nitrogen to phosphorus has previously been found to influence cyanobacterial growth and dominance in ecological systems (Smith, 1983). The ratio of total N:P appeared to be lower when *L. majuscula* was blooming, as opposed to other times at both NDB and EMB (Figure 4.13 and Figure 4.14).

4.3.9 Environmental Contamination with *L. majuscula* Toxins

On 22/1/01 8 L of seawater was collected at Pebble Beach, 200m from shore, in an area with large amounts of *L. majuscula* growth (>90% cover and >10cm thickness). Both LA and DAT were below detectable limits. Similarly, 2 kg of sand collected from underneath wracks of *L. majuscula* washed onto Pebble Beach, NDB did not have any detectable amounts of LA or DAT. No saxitoxin was found in samples of Queensland derived *L. majuscula*. Air samples were collected using an Environmental Protection Agency, Queensland Government high volume air sampler in Deception Bay. No trace of toxins of *L. majuscula* or fibres were found on filters after 24 hrs. Detection limits of analysis for both LA and DAT was 40 ng/ml.

4.3.10 DAT and LA in Hawaiian *L. majuscula*

Samples of *L. majuscula* were obtained from open ocean waters near Honokowi, West Maui, Hawaii. Both DAT and LA were detected in samples, but LA ranged between 10.4-275.5 mg/kg, approximately thirty times greater than the level of DAT (ranged between 0.03-0.84 mg/kg). Levels of LA were the highest recorded in this study at 275.5 mg/kg.

4.3.11 DAT and LA in *Lyngbya wollei*

Samples of *L. wollei* were obtained from Florida in conjunction with John Burns, Cyanolab, Palotka, Florida and Florida State Health Department. A sample from a lake in Polk County was found to have a reading of LA 3.8±0.6 mg/kg although other samples were below detectable limits.

4.4 Environmental Toxicology - Discussion

4.4.1 Introduction

An examination has been made to determine if *L. majuscula* exhibits differential temporal and/or spatial toxin levels of DAT and LA in Moreton Bay. An attempt was also made to examine if environmental factors influence levels of DAT and LA in *L. majuscula*. Little is known about effects of environmental parameters on the growth and/or toxin levels of *L. majuscula*, primarily because culture of individual strains has not been achieved, or has not been fully utilised (Rossi *et al.*, 1997; SEQRWQMS, 2001; Burja *et al.*, 2002). Examining factors in the field is difficult, as several variables are often intricately interrelated; however the advantage is that these are ecologically relevant samples.

4.4.2 Temporal and Spatial Differences in Toxin Levels

Definite spatial and temporal differences have been noted in *L. majuscula* growing in Moreton Bay. A trend of either LA or DAT being produced preferentially by single samples was noted. Samples from Hawaii and Moreton Bay showed the ability to produce both toxins. Interestingly, samples from NDB produced only DAT while samples from EMB produced LA in the majority of cases. Causes of this difference in toxin production can be postulated as either genetic or environmental. Little is known about the genetic diversity of this species, although research is under way (Paul, 2001; SEQRWQMS, 2001; Salmon *et al.*, 2002). Both molecules are composed of C, H, O and N and trace elements are not present in the toxins. Probable factors influencing production of these "secondary metabolites" are activities of metabolic pathways preceding the synthesis of these compounds. Similar levels of

either toxin produced (similar orders of magnitude) suggest similar ecological tasks, such as feeding deterrents.

4.4.3 Bloom Size and Toxin Levels

Samples collected when the blooms reached their maximum area and density contained higher toxin concentrations on a dried weight basis than when blooms were first initiated, both at NDB and EMB, with DAT and LA respectively (Figure 4.3 and Figure 4.4). Orr and Jones (1998) have previously shown that the production of microcystin by cyanobacteria in culture is directly proportional to the rate of growth, irrespective of the environmental conditions. This has been further refined so that the cellular microcystin content of N-limited *Microcystis aeruginosa* can be predicted from growth rate (Long *et al.*, 2001), and faster growing cells contained higher intracellular concentrations of microcystins. Sivonen (1996) also noted that cyanobacteria produce most toxins under favourable growth conditions.

Our recent data have shown toxin levels of both LA and DAT correlate with carbon, nitrogen and phosphorus levels in *L. majuscula.* Assuming that higher levels of these elements indicate a healthy environment for growth, these data support the finding that toxin levels were at their highest at the height of the bloom, when presumably the organism had best conditions for growth. This is supported by levels of nutrients matching Redfield's ratios, indicating balanced growth (O'Neil, pers. com.). An alternative hypothesis is that the presence of a moderate correlation between Cu and S and toxin levels, possibly present from runoff from the catchments area (Clark *et al.*, 1997), may indicate a stress response to these elements, known to have detrimental effects on cyanobacteria (Brand *et al.*, 1986; Dyer *et al.*, 1992).

Table 4.4 Correlation between toxin levels (DAT or LA) and nutrient level.

	R^2
P	0.76
N	0.76
K	0.75
C	0.73
Cu	0.64
S	0.54
B	0.47
Na	0.24
Mg	0.17
Al	0.09
Ca	0.09
Fe	0.07
Mo	0.05
Mn	0.01
Zn	0.00

4.4.4 Differences in Toxin Production in a Population

Much of the work on cyanobacteria toxin regulation by environmental conditions has been conducted using freshwater cyanobacteria. Environmentally induced changes in toxin quotas are usually in the range of 3-4 fold (Sivonen and Jones, 1999). Although significant, these differences can be better put in context when compared with the three orders of magnitude range in toxin content found between different strains of the same species (or at least morphologically similar) (Bloch *et al.*, 1997). Toxic and non-toxic strains of cyanobacteria have been found to grow together. It has been reported that higher concentration of toxins are due to the differential growth of individual strains, not necessarily with differing morphological characteristics, within a population (Sivonen and Jones, 1999). It has also been suggested by some that plasmids may be involved in toxin production (Vance, 1977; Schwabe *et al.*, 1988; Gallon *et al.*, 1994), although this theory has been disputed by

others (Bloch *et al.*, 1997). If this is correct there is a possibility of plasmid transfer and therefore transfer of toxin production capability between individual organisms in a population (Waldor and Mekalanos, 1996; Jiang and Paul, 1998).

Differences have been found in the concentrations of DAT at a single site with levels ranging from 0-11.17 mg/kg FDW. These differences are in the range of one order of magnitude where the range of toxin levels of *L. majuscula* in Morton Bay has ranged to two orders of magnitude. These samples of *L. majuscula* grew under similar environmental conditions, assuming no micro-environmental differences. This suggests genetic differences may only be partially responsible for differences in toxin levels.

4.4.5 Physical Parameters

L. majuscula toxin levels were highest during the summer periods when light intensities were at there highest. Cyanobacteria (*Microcystis* spp.) have been shown to have the lowest production of toxins at low light intensities (2-20 μmol photons $m^{-2}s^{-1}$) and highest between 20 and 142 μmol photons $m^{-2}s^{-1}$ (Kaebernick and Neilan, 2001). Strains (including *Microcystis, Nodularia, Oscillatoria, Anabena* and *Aphanizomenon*) examined by other authors have also been found to produce most toxins when growing at "optimum" light conditions (Sivonen, 1990; Rapala *et al.*, 1993b; Sivonen and Jones, 1999). Watkinson (2000) reported a prolonged period of high light for the initial bloom period of *L. majuscula* in NDB in January 2000. This bloom was later to be found toxic. The average surface light was recorded as 517.36 μmol quanta $m^{-2}s^{-1}$ with a low light attenuation coefficient, averaging at 0.78 m^{-1}, with average water depth of 1.5 m in the NDB bloom area. The quantity of light available to cyanobacteria in Moreton Bay is in the range for high toxin production in

cyanobacteria. The presence of iron is also important when considering light intensities as high light intensities increase iron uptake and this may lead to higher toxin production (Utkilen and Gjolme, 1995), although this has been disputed by other authors (Lukac and Aegerter, 1993; Lyck *et al.*, 1996). In general, the role of iron in levels of cyanobacterial toxins remains unclear (Sivonen and Jones, 1999). *L. majuscula* has shown a distinct growth dependence on Fe (Gross and Martin, 1996).

Turbidity (Secchi depths) of the water at sites in NDB and EMB were highest for the year during the time of high light intensity and temperature. Waters were relatively clear, if depth of these areas is taken into account, with NDB ranging from 1-4 m and EMB ranging from 2-5 m.

Also linked to light intensity in the shallow waters of Moreton Bay is water temperature. Water temperature was closely related to air temperature in Moreton Bay, both increasing from the end of June. Blooms began when water temperature was over 25°C in NDB and EMB. Watkinson (2000) found that water temperatures increased to between 24°C and 25°C before a bloom event in DNB in 2000. Bloom formation in the marine cyanobacterium *Trichodesmium* has been reported at temperatures above 21°C (Sellner, 1997). Optimum temperature for cyanobacterial growth has been reported to be above 25°C (Fogg *et al.*, 1973; Robarts and Zohary, 1987), which are higher than for green algae and diatoms (Mur *et al.*, 1999).

In general, cultured cyanobacteria *(Microcystis, Aphanizemenon, Nodularia* and *Anabena*) have been found to have maximal toxin concentrations when water temperatures are approximately 25°C, coinciding with the temperature for maximal growth (Jones and Sivonen, 1999). Lowest toxin concentrations were found at 10°C and 30°C. Cyanobacteria that have been grown at different temperatures have also been shown to produce different toxins (Rapala *et al.*, 1997). This may be a reason

for the difference in toxin being produced between NDB and EMB, although temperatures differed only by small amounts (1-2°C).

4.4.6 Weather Conditions

Stability in water column has been found advantageous in growth of marine macroalgae (Hurd, 2000), as well as cyanobacteria, and may be a more important influence on abundance and distribution of cyanobacteria than nutrient availability (Carpenter, 1983; Capone, 1997; Cheroske *et al.*, 2000). Water motion affects a variety of factors influencing algal production including photon flux density and spectral composition, nutrient availability, temperature, inter- and intra-specific competition for space and resources and rates of herbivory (Hurd, 2000). The monthly average direction of maximal wind gusts of 125° (ESE) in the period leading up to the 2001 bloom compares favourably to data collected in 2000 (Watkinson, 2000). Wind from this direction would result in maximal water motion due to the positioning of Bribie Island and the mainland with wind having an uninterrupted run across Moreton Bay (Figure 4.1).

The pH for both NDB and EMB was in the range for normal sea water (pH 8-8.3) and normal sediments (pH 7-8) (Mirlean *et al.*, 1999). Similar ranges of pH have been reported for estuaries nearby on the Eastern Australian seaboard (Williams, 1987).

4.4.7 Nutrients and Oxygen

A gradient of P and N exists from highest in the rivers, then lower in the estuaries and lower again in the Western Bay area and finally lowest in EMB (Dennison and Abal, 1999). After rain events, a spike in nutrients entering Moreton

Bay from the catchments area could be predicted. A large rain event (107mm) occurred on 2nd February 2001 and subsequently spikes in nutrient levels occurred, especially in the estuarine NDB (Figure 4.15). These have previously been found to elicit a response in phytoplankton communities (Heil *et al.*, 1998). Runoff events bring in organic matter that binds Fe and P, which may deliver these essential nutrients in a bioavailable form (Albert, 2002; O'Neil *et al.*, 2002). Blooms of toxic *Trichodesmium* have been noted after unusual enrichment of the coastal waters by drainage from the land (Sato, *et al.*, 1966)

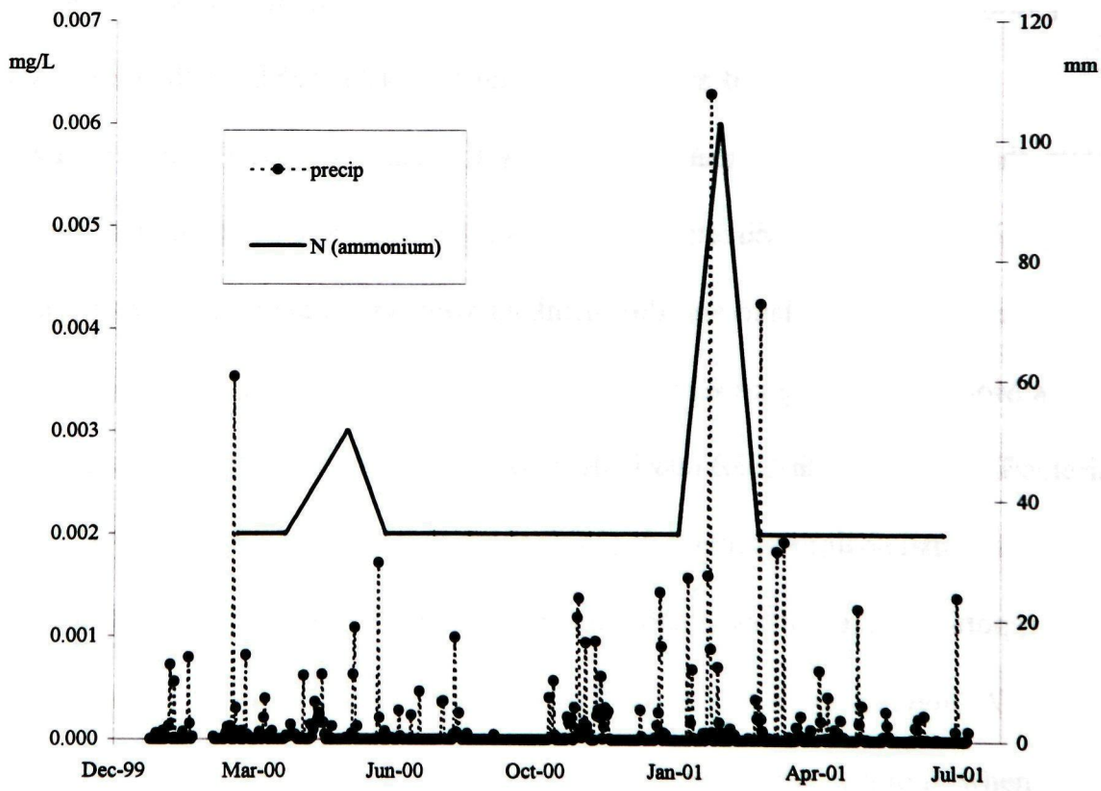


Figure 4.15 Relationship between precipitation and N (ammonium) at NDB.

The presence of nitrogen, phosphorus or oxygen did not appear to affect *L. majuscula* growth or toxin production in general. Other studies have shown that the epiphytic algal communities are not nutrient limited in areas of the Queensland coast (Koop *et al.*, 2001). In contrast, Kuffner and Paul (2001) found that in the Cocos Lagoon, Guam, *L. majuscula* appeared to have increased growth in response to phosphate in the water column, as well as having more efficient growth and/or nutrient uptake systems than other species. Thacker and Paul (2001) concluded that physical disturbance can be more important influence on cyanobacterial abundance and distribution than either nutrient availability or interactions with macroalgae. An increase of N (ammonium) after the bloom would be expected from decay and lysis on the biomass, and may add a substantial amount of N to the system (Gonzalez and Suttle, 1993).

In lakes, P is usually the limiting nutrient and hence leads to reduced toxin production by arresting growth (Kaebernick and Nielan, 2001). Alternatively, low concentrations of P may decrease toxin production, as has been found in the case of microcystin (produced by *Anabena*, *Microcystis* and *Oscillatoria*), anatoxin-a (produced by *Aphenizomenon*) and nodularin (produced by *Nodularia*) (Jones and Sivonen, 1999).

The ratio of total N:P appeared to be lower when *L. majuscula* was blooming, as opposed to other times at both NDB and EMB (Figure 4.13 and Figure 4.14). This mirrors findings of Schindler (1977) and Smith (1983) that found the dominance of cyanobacteria in occurred in freshwater lakes when N:P ratios were low.

4.4.8 Biotic Parameters

Peaks in the amounts of chlorophyll-a measured were recorded during bloom episodes of *L. majuscula.* Blooms of phytoplankton are to be expected with the presence of nutrient source(s) (Dennison and Abal, 1999). Presence of large numbers of photosynthetic organisms in the water column suggest ideal growth conditions were present for planktonic photosynthesisers were present at the same time that ideal growth conditions for the benthic *L. majuscula.* This suggest at both sites in Moreton Bay the environment for growth of photosynthetic organisms was at its most favourable at the first week of February 2001 at NDB and first week of March at EMB. A trend of blooms occurring first at NDB has been found over several years (2000, 2001, 2002), followed by EMB. The maximum intensity of blooms also occurs earlier in NDB than EMB. This could be due to several factors such as proximity to catchments areas, water depth, tidal flow and sheltering from landmasses.

Pheopigments are the degradation products of chlorophyll-a that are present when zooplankton are grazing on photosynthetic organisms such as phytoplankton. Zooplankton may have been present in NDB during the time of the bloom, and had grazed on higher amounts of chlorophyll-a containing organisms, releasing pheopigments. The size of the *L. majuscula* bloom decreased soon after the first measurement of high pheopigment concentrations and perhaps high numbers of grazing zooplankton. Zooplankton may have been preferentially feeding on phytoplankton that were in competition with *L. majuscula* for nutrients. Feeding pressure on zooplankton by other marine organisms may also lead to increase in release of nutrients that could be taken up by cyanobacteria (Burns, 1987).

4.4.9 Presence of Extracellular Toxins in Environment

Levels LA or DAT were below detectable limits when measured in both water surrounding dense algal blooms and in sand from the beach covered in wracks of cyanobacteria. It has been reported that ultraviolet light may degrade compounds responsible for the toxicity of *L. majuscula.* Hashimoto (1979) records reports of half of the toxicity being lost after three hours exposure to ultraviolet radiation. Anecdotal evidence suggest some toxicity remains once *L. majuscula* has been dried, as witnessed by fishers reporting eye and respiratory irritation when removing cyanobacteria from nets (Dennison and Abal, 1999). *Lyngbya aeruginosa-coerulea* has been found to release substantial amounts of cytotoxic compounds into the surrounding medium, and these were probably not due to cell lysis as cytotoxic effects differed between cells and medium (Treneva *et al.*, 2003).

For a variety of cyanobacteria, it has been found that during healthy log phase cultures 10-20% of toxin is extracellular (Jones and Sivonen, 1999). Other cyanobacterial toxins such as microcystin have been found to persist in the environmental after they have been released from cells. In the field, where the bloom (*Microcystis aeruginosa*) is not obviously degrading, dissolved cyanotoxins are in the range of 0.1-10 μgL^{-1} (Jones and Sivonen, 1999). Microcystins have been found to persist as dissolved and particulate toxin, with 30 and 15 days respectively required for 90 percent degradation to occur (Lahti *et al.*, 1997). The use of algaecides such as copper sulphate has in the past lead to lysis of cyanobacteria and release of toxins in water (Jones and Sivonen, 1999).

Microcystins have previously been found to attach to suspended solids in rivers and reservoirs, but no more than 20 percent of the total concentration. Rapala *et al.* (1993a) reported dissolved anatoxin-a and microcystins were attached to lake

sediments. The potential biodegradation of toxin contained in *L. majuscula* by UV light, bacteria or other organisms is yet to be explored. With the tidal nature of the estuary at North Deception Bay rapid dilution and dissemination of toxins would be expected, especially if wind action is also taken into account.

No LA or DAT or filaments were discovered after using a high volume air sampler for 24 hours. This was not an unexpected outcome as aerosolisation of *L. majuscula* has only rarely been reported (Izumi and Moore, 1987; Anderson *et al.*, 1988), and requires specific meteorological conditions which may be rare in Moreton Bay due to presence and absence of various geographical parameters.

4.4.10 Presence of Toxins in other species of *Lyngbya*

A single sample of *L. wollei* was found to contain LA. This has previously been unreported and further studies into this phenomenon are underway. Similar toxins occurring in different genus' of cyanobacteria has been recorded previously. Saxitoxins originally found in cyanobacteria in the species *Aphanizomenon flos aquae* (Mahmood and Carmichael, 1986a) but were later found to occur also in *L. wollei* (Carmichael *et al.*, 1997). Similarly, microcystins have been found in *Microcystis*, *Anabaena*, *Oscillatoria*, and *Nostoc* species. Recently another member of the genus, *Lyngbya aeruginosa-coerulea* has been found to be both neuro- and hepato-toxic, although no microcystins or saxitoxins were present (Treneva *et al.*, 2003). Presence of LA in *L. wollei* will possibly have public health implications with large numbers of freshwater bodies in Florida as well as other states in the USA have this organism present. These areas are used for both recreational and drinking water.

4.4.11 Conclusions

- LA and DAT occur at different concentrations in different geographic location.
- LA or DAT may be absent altogether.
- Ten fold differences in concentration occur over small distances (<2 m).
- Highest concentrations of toxin occurred when bloom size and density were also at their maximum.
- Bloom intensity occurred when water temperature was maximal.
- Periods of low precipitation preceded blooms.
- Peaks of chlorophyll-a were seen during periods of high bloom intensity
- *L. wollei* has been found to contain LA.
- Close correlation was found between toxin levels and concentrations of C, N, P and K in *L. majuscula.*

5. Public Health and *Lyngbya majuscula*

5.1 Introduction

The marine cyanobacterium *Lyngbya majuscula* has been implicated in acute adverse health effects in humans over the last forty years (Grauer and Arnold, 1961; Izumi and Moore, 1987). Anecdotal cases have come to the attention of researchers in South East Queensland during the last 5 years (Dennison *et al.*, 1999; Abal *et al.*, 2001). Presence of this organism has led to erection of warning signs and beach closures in SE Queensland and the establishment of a "*Lyngbya* Management Strategy", mirroring the general world-wide trend of increasing beach closures and reported illness after exposure to marine water (Havell *et al.*, 1999). A similar rise in the last three decades of marine algal toxic incidents has occurred (see van Dolah, 2000; Henrickson *et al.*, 2001 for reviews).

Toxic cyanobacteria are responsible for almost all known cases of fresh or brackish water intoxications involving phycotoxins (Carmichael, 2001). Although some studies have been conducted examining the effect of cyanobacteria on human health (Elder *et al.*, 1993; Bell and Codd, 1994; Falconer, 1994; Ressom *et al.*, 1994; Codd *et al.*, 1999; Kuiper-Goodman *et al.*, 1999; Chorus *et al.*, 2000; Pitois *et al.*, 2000; Carmichael, 2001 for reviews), examination of the effects of cyanobacteria on human populations has primarily been explored in the freshwater arena (Philipp, 1992; Philipp *et al.*, 1992; el Saadi *et al.*, 1995; Pilotto *et al.*, 1997; Pilotto *et al.*, 1999; Stewart *et al.*, 2001; Torokne *et al.*, 2001). Retrospective studies have been conducted after toxic incidents involving *L. majuscula* in the marine environment (Grauer, 1959; Grauer and Arnold, 1961; Serdula *et al.*, 1982; Anderson *et al.*, 1988). It has been difficult to compare the situation in Queensland with these studies as they involved low numbers of people exposed, differing routes of exposure, and since

those conducted forty years ago, there have been major changes in marine recreational water activity.

Symptoms from exposure to freshwater cyanobacteria have generally concerned liver and central nervous system health outcomes due to the predominance of hepatotoxic and neurotoxic compounds present in these organisms. Less common are reports of episodes of dermatitis after exposure to cyanobacteria but these include both allergenic and non-allergenic effects (el Saadi *et al.*, 1995; Torokne *et al.*, 2001). It is currently unclear as to whether these symptoms are due to toxins present in the cyanobacteria, the lipopolysaccaride in the outer layer of the cell wall, or a combination of these.

Skin complaints after marine recreational water activity (MRWA) have been allocated individual terms based on differential diagnoses. (Izumi and Moore, 1987) used the phrase "seaweed dermatitis" to delineate *L. majuscula* dermatitis from that caused by other organisms (Table 5.1).

Table 5.1 Differential definitions of dermatitis after water activity.

	hydroid dermatitis	**swimmer's itch**	**seabather's eruption**	**seaweed dermatitis**
water	salt	usually fresh	salt	salt
area on body	exposed	exposed	covered	covered
cause	nemocysts	schistosome	planula larvae	*L. majuscula*

(Source : Fisher, 1995; Wong *et al*., 1994)

Fisher (1995) defines "swimmers itch" as pruritic dermatoses associated with exposure to bodies of water with no identifiable cause and uses schistosome cercarial dermatitis in place of swimmers itch (Fisher, 1995).

Symptoms from exposure to toxic *L. majuscula* include dermatitis involving

itching, rash, burning blisters and deep desquamation, causing pain (Solomon and Stoughton, 1978), respiratory irritation (Anderson *et al.*, 1988) and more rarely burning of the upper gastrointestinal tract (Marshall and Vogt, 1998). These different routes of exposure to *L. majuscula* can all occur when human populations have acute exposure to episodic blooms. It has been shown that the toxins lyngbyatoxin A (LA) and debromoaplysiatoxin (DAT), isolated from *L. majuscula* samples in Hawaii (Mynderse *et al.*, 1977; Cardellina *et al.*, 1979) and Okinawa (Fujiki *et al.*, 1985), are at least partially responsible for these symptoms (Solomon and Stoughton, 1978).

L. majuscula has been found to grow in more than 98 locations around the world in tropical, sub-tropical and temperate climates. In Australia, it has been identified in Hervey Bay, Fraser Island, Moreton Bay, Bundaberg, Hardy Reef Lagoon (Queensland sites) and Peel Inlet (Western Australia sites). The presence of *L. majuscula* in North Deception Bay was first reported in a scientific taxonomic study during the early 1960s (MBWCP, 2002). Since 1997 *L. majuscula* blooms have been reported in Moreton Bay, with outbreaks occurring in two distinct locations, Southern Pumicestone Passage/North Deception Bay (NDB) and on Amity and Moreton Banks, in the Eastern Moreton Bay region (EMB). Major blooms were recorded in the summers of 1999/2000 (25-30 square kilometres in EMB) and 2000/2001 (10 square kilometres in NDB)(MBWCP, 2002). LA and DAT have been found in *L majuscula* samples from Moreton Bay, Queensland (Osborne *et al.*, 2001b; Osborne *et al.*, 2001c; Osborne *et al.*, 2001d).

The aim of this study was to investigate the frequency and severity of injuries associated with exposure to the cyanobacterium, *L. majuscula*. Two approaches were undertaken. Firstly an examination was made of the health status of recreational water users of seaside residential areas of Bribie Island and environs, an area where

blooms of the toxic cyanobacterium *L. majuscula* occur ('Bribie Island Study'). Anecdotal evidence had suggested that individuals had been experiencing both high levels and severity of symptoms due to exposure to *L. majuscula* and its associated toxins. These symptoms have primarily fallen into the skin, eye and respiratory irritation categories (Dennison *et al.*, 1999).

A secondary aim was to establish if there were increased risks of adverse health outcomes in specific groups within the population. Specific groups and behaviours within the population were assessed to examine if any specific groups or their behaviours might differentially increase risk of adverse health outcomes after exposure to toxic *L. majuscula.* Finally, the study was designed to allow an assessment of the public health importance of *L. majuscula* in South East Queensland, and the possibility for public health intervention.

Secondly, in a retrospective analysis of first-aid records from Fraser Island, Queensland, records from years when blooms of *L. majuscula* were known to have occurred were compared to records from years when *L. majuscula* was not present ('Fraser Island Study').

5.2 Methods – Bribie Island Study

5.2.1 Ethical Considerations

Ethical approval for this study was sought and gained from the Behavioural and Social Sciences Ethical Review Committee, University of Queensland (approval number B/169/NRCET/SOCPREVMED/99/PHD). The questionnaire followed guidelines laid down by the National Health and Medical Research Council (NHMRC) (NHMRC, 2000) and the International Epidemiology Association/European Epidemiology Group (Bolumar *et al.*, 2002).

5.2.2 Study Design

A range of methodologies was considered prior to the decision to attempt a study. These were assessed and ranked on the basis of cost verses value of information derived, and speed of recovery of this information. Previous researchers examining the health effects of cyanobacteria during recreational water activity had used a prospective-cohort methodology (Philipp, 1992; Philipp *et al.*, 1992; Pilotto *et al.*, 1997). A pilot study with a similar protocol was attempted in this study but low participation rates, low exposure and/or presentation of symptoms, and poor predictability of the presence of *L. majuscula* made this approach unfeasible (Figure5.1). Persons interviewed had either heard of *L. majuscula* and knew to avoid it, or had not heard of *L. majuscula* and were not interested. Neither group was interested in taking part in interviews. Furthermore, unlike planktonic cyanobacteria that are dispersed relatively homogeneously through the water column, benthic cyanobacteria such as *L. majuscula* have a tendency to form clumps due to their macroscopic nature, and this puts them much more under the control of the meteorological conditions. The unpredictable nature of the presence of *L. majuscula*

meant it was unfeasible to attempt a survey such as those attempted by Pilotto *et al.*, (1997) as the presence of *L. majuscula* at a sampling site could not be guaranteed day to day.

Based on previous experience, *L. majuscula* blooms were known to occur in the Bribie Island area. Phone calls were made to the five pharmacies on Bribie Island and it was asked how many people per year approached each pharmacist for treatment of *Lyngbya*-like symptoms. The totals indicated that sufficient numbers of individuals with symptoms severe enough for them to attend a pharmacist would be present to warrant a larger health survey of this area. It was decided to attempt a cross-sectional epidemiological study. The survey period covered seven months of 2001, from January to July. This period covered a time when blooms of toxic *L. majuscula* had previously occurred, when water temperatures are higher.

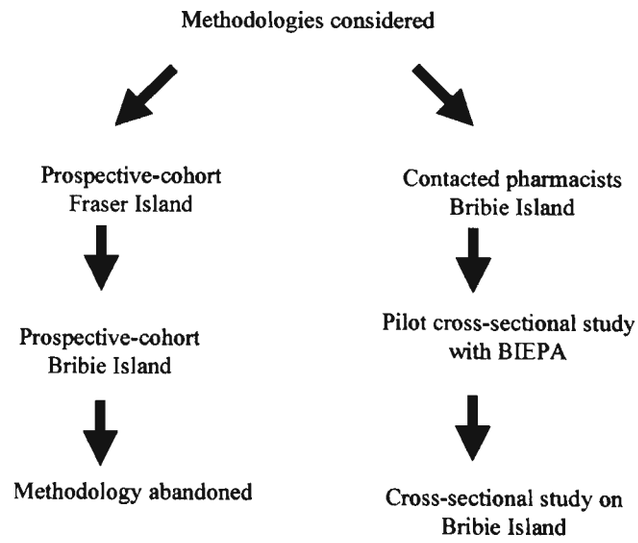


Figure 5.1 Flow chart of decision making process on which study type to attempt (BIEPA = Bribie Island Environmental Protection Association).

5.2.3 Questionnaire Development

The questionnaire (see attached instrument in appendix) was developed using guidelines provided by a number of sources including (World Health Organisation, 1990; Ressom *et al.*, 1994; World Health Oraganisation, 2000). Similar questions have previously been used to examine the effects of cyanobacteria on the health of recreational water users (Philipp *et al.*, 1985; Philipp, 1992; Philipp *et al.*, 1992; Pilotto *et al.*, 1997; Pilotto *et al.*, 1999).

Questions asked of participants can be divided into six main groups. Participants were asked questions initially on what type of water activity they undertook, if any, in Moreton Bay waters in the previous seven months. In an attempt to get an estimation of the extent of exposure, questions were asked on the types and duration of water exposure. Participants were asked which of fifteen recreational activities they had undertaken. These were later divided into low, medium or high exposure groups. Participants who reported symptoms were also asked about the duration of water exposure, with choices of less than thirty minutes, thirty to sixty minutes and greater than sixty minutes given.

Participants who reported water exposure were asked if they had suffered any of 17 symptoms commonly associated with exposure to cyanobacteria and/or toxins associated with *L. majuscula*. They were further asked to specify whether symptoms were mild, moderate or severe in nature.

Those who reported symptoms were asked further questions on the types and expense of treatment sought. All participants were asked if they had experienced any breathing problems or symptoms of aerosolised *L. majuscula*. Finally, a series of

questions were asked to ascertain participants' knowledge of, and attitudes towards *Lyngbya* and exposure.

The questionnaire was trialled by members of the Bribie Island Environmental Protection Association (BIEPA). The questionnaire was included in the monthly newsletter of BIEPA after the author had given a presentation to BIEPA members. This newsletter has a circulation of 300 members. With a return rate of 23% among what was considered a potentially 'user-friendly' group it was decided to send a follow-up postcard as an attempt to increase return rates.

5.2.4 Subject Selection

Unlike other areas in Moreton Bay where blooms occur, the Bribie Island area has a high population density (ABS, 2001). The target population was marine recreational water users who lived on Bribie Island and nearby suburbs. Extrapolation of rough estimates of people with *Lyngbya*-like symptoms attending pharmacists (0.2% of Bribie Island population) suggested that 1000 replies would be required to see significant numbers of individuals with symptoms. Assuming a response rate of 20%, it was estimated that 5000 surveys would need to be posted, which was achievable with the available resources.

Subjects to whom questionnaires were addressed were selected from the Australian Electoral Commissions electoral roll for the Federal Division of Longman (Australian Electoral Commission, 2001). Subjects were selected only from those whose home addresses were in the suburbs of Banksia Beach, Bellara, Bongaree, Godwin Beach, Sandstone Point, White Patch and Woorim. These corresponded to all suburbs in the 4507 postcode area and partially in the 4511. Percentages living in each suburb and percentage of age group in that suburb were calculated. Data from

the Australian Electoral Commission came in age groups of five-year cohorts (18-22, 23-27, 28-32, 33-37, 38-42, 43-47, 48-52, 53-57, 58-62, 63-67, 68-72, 73-77, 78-82, 83-87, 88-90). Names and addresses were randomly selected, by random number generation, so that each suburb would be represented in proportion and each age group within that suburb was also in proportion.

5.2.5 Questionnaire

A self-administered questionnaire was posted to 5000 people requesting participants. On Friday 20th July 2001, 5000 survey forms were posted in individually addressed letters, together with reply paid self-addressed envelope. Postage stamps were not used for economic reasons, and because no difference had been found in their ability to increase response rates (Harrison *et al.*, 2002). Four weeks later a reminder postcard was sent to those who had not replied. This reply paid self-addressed postcard asked six questions of the original addressee, which were answered by ticking a box.

These included:

I never received a survey - send me a survey!

I have replied

I intend to reply

I have lost my survey - send me another survey!

I wish to be part of the survey - send me a survey!

I do not wish to be part of the survey.

No support from the local news media was sought or received in relation to this questionnaire, despite the volatile nature of this issue in the area, as it was felt this might bias the survey.

5.2.6 Data Entry and Data Cleaning

A research assistant, Wendy Shaw, entered data for approximately 200 surveys. The author entered the remainder. All data were initially entered into a Microsoft Access spreadsheet (Microsoft, 1997a). Data were assessed for any replication of records. Data were then copied to SPSS (SPSS, 2002) for analysis. Some analyses were conducted using Epi Info (Dean *et al.*, 1995). Tables and graphs were created in Microsoft Excel (Microsoft, 1997b). Frequency tables were constructed using SPSS and data was checked examining ranges and distributions.

5.2.7 Statistical Analysis

A variety of statistical analyses were made in this thesis. χ^2 tests were conducted using SPSS for categorical variables to test if observed frequencies did not differ from expected frequencies (SPSS, 2002). T tests were conducted using SPSS to compare means of normally distributed continuous variables (SPSS, 2002). The Epi Info program was used to calculate Mantel-Haenszel summary χ^2 to examine a dichotomous response variable conditional upon covariate pattern defined by one (control) variable (Dean *et al.*, 1995).

Odds ratios and 95% confidence intervals were calculated using logistic regression in SPSS (SPSS, 2002). Logistic regression was selected as a statistical method as it allowed for the adjustment for potential confounders. It also allowed examination of the association between symptoms and exposure, and the potential

effects of age and sex. Having or not having a symptom is a dichotomous variable and therefore well suited to logistic regression. Predictor variables of age, sex and exposure are a mixture of continuous and categorical variables, better suited to logistic regression than discriminant variable analysis or logit analysis.

5.3 Results - Bribie Island Study

5.3.1 Response

Of the 5000 surveys posted to residents of North Deception Bay (NDB), 1370 survey forms were returned, representing a response rate of 27.4%. The response was aided by sending out a reminder postcard four weeks after sending out the original survey (Figure 5.2). As the survey was anonymous it was not possible to calculate the effect of the reminder postcard on survey return but the numbers of returned surveys increased after the reminder postcards were sent out. Out of 3850 reminder postcards sent, 959 (24.9%) were returned, but only 176 (4.6%) requested a questionnaire form to take part in the survey (Table 5.2). Further follow up was not conducted due to ethical and financial considerations.

Table 5.2 Response to reminder postcard.

	no.
asked for a survey	176
had already replied	89
wrong address	71
did not wish to be part of the survey	618
deceased	5
Total	959

In an attempt to establish the similarity between the population residing in NDB and that replying to the UQ questionnaire, several comparisons were made. As can be seen from Table 5.3, several similarities and differences exist. It should be noted that ABS statistics for Bribie Island (ABS, 1996) do not include the two suburbs on the mainland also included in this survey, the 86 persons at Godwin Beach (Australia Post postcode 4511) and 614 persons at Sandstone Point (Australia Post postcode 4511).

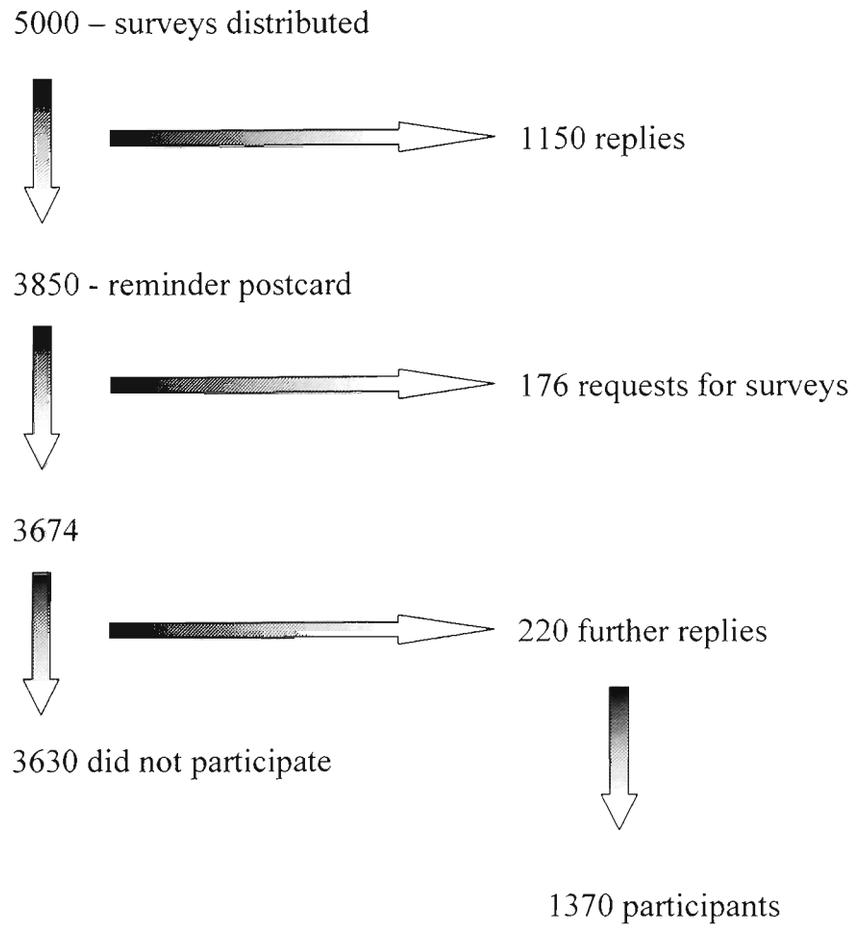


Figure 5.2 Flow diagram of postal survey responses.

Bribie Island is a retirement area and this is reflected in the high average age of both the UQ study population (57.2 years) and the ABS study population (eighteen years and over) (55.0 years) (ABS, 1996). The average age in Deception Bay, the neighbouring ABS area, was 42.3 years for the 18 years and over age group (ABS, 1996). A similar age distribution was seen between the study population and ABS data for Bribie Island (Figure 5.3) with the majority of people aged between 45 and 74. Fewer surveys were returned in the younger and older age groups when compared

to the numbers of people in these age groups found in the ABS survey (Figure 5.3). The difference was greater in the older (75-90) age group.

A slightly higher proportion of males (52.1%) responded to the survey than females when compared to the ratio recorded by the ABS (χ^2, $p < 0.05$) (Table 5.3). The surveyed population records high numbers of retired persons and persons listed as homemaker (combined total 63.5% of population 18 years and over). Several measures of the workforce have been made. The civilian population consists of two mutually exclusive groups, the labour force, and persons not in the labour force. The labour force consists of those that are employed and those that are unemployed. The proportion of unemployed persons was significantly lower in the UQ survey when compared to the ABS statistics for Bribie Island (4.0% verses 17.9%, $\chi^2 < 0.05$).

The proportion of the study population who reported current smoking (14.8%) was significantly lower than the national statistic of 23.8% (ABS, 1998) (χ^2, $p < 0.05$). The proportion of individuals suffering asthma recorded in the UQ survey (14.4%) was significantly higher than the 11.3% recorded by the ABS in 1995 (ABS, 1998) (χ^2, $p < 0.05$) (Table 5.3).

Table 5.3 Comparison of study population to population as recorded by ABS.

		UQ Study Pop	Bribie Is	Australia
population size	no.	1,370	12,946	19,600,000
age	mean (years)	57.2	55.0[#]	
sex	male	52.1%	47.9%[#]	47.9%*
	female	47.9%	52.1%[#]	52.1%*
labour	survey size	1,370	10,814[§]	13,646,666[Φ]
	not in labour force	63.9%	63.5%[§]	27.1%[Φ]
	in labour force	36.1%	36.5%[§]	72.9%[Φ]
labour force	employed	96.0%	82.1%[§]	92.1%[Φ]
	unemployed	4.0%	17.9%[§]	7.9%[Φ]
smoking habits (current)	no	85.2%		76.2%^
	yes	14.8%		23.8%^
asthma		14.4%		11.0%[α]

[#]ABS, aged 18 and over, 1996

*ABS, citizens aged 18 and over, 1996

[§]ABS, aged 15 years and over, 1996

[Φ]ABS, 15-69 years, 2001

^ABS, aged 18 and over, 1995

[α]ABS all ages, National Health Survey, 1995

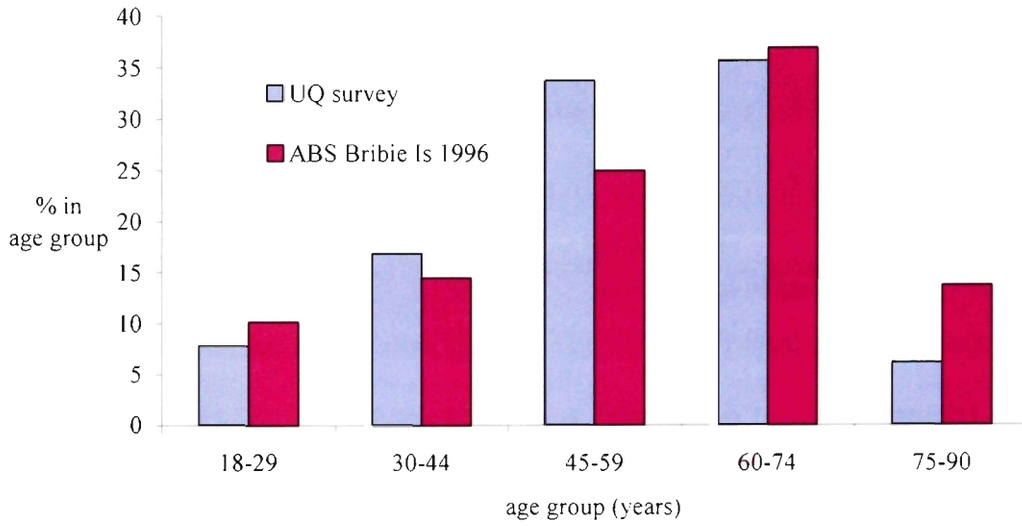


Figure 5.3 Age distribution of participants in UQ "Health of Ocean Users Survey" compared to ABS statistics for Bribie Island (ABS, 1996).

5.3.2 Population Undertaking MRWA

High numbers of people responding to the survey reported marine recreational water activity (MRWA) in the past seven months (78.2%). The average age of those reporting engaging in MRWA was 54.5 years (n = 1065) compared to 66.8 years (n = 287) for those reporting no water contact (t test, p=0.000). The trend was for older age groups to undertake less MRWA. The percentage fell from 97.2% at age 18-29 having contact with the water to 37.8% at age 75-90 (χ^2, p=0.000). A slightly higher proportion of male respondents (80.3%) undertook MRWA than females (75.9%) (χ^2, p = 0.06). A higher proportion of current smokers than non-smokers undertook MRWA (χ^2, p = 0.001) however this difference disappeared after adjusting for age (Mantel-Haenszel summary χ^2 was p = 0.175).

Table 5.4 Proportion of the study population undertaking MRWA by age, sex and smoking status.

	groups	MRWA		no MRWA		test	p
		no.	(%)	no.	(%)		
age	18-29	103	97.2	3	2.8	χ^2	0.000
	30-44	215	94.3	13	5.7		
	45-59	378	83.1	77	16.9		
	60-74	338	70.3	143	29.7		
	75-90	31	37.8	51	62.2		
sex	male	569	80.3	140	19.7	χ^2	0.060
	female	494	75.9	157	24.1		
current smoking	no	828	76.6	252	23.4	*χ^2	0.175
	yes	166	88.3	22	11.7		

(*Mantel-Haenszel pooled estimate χ^2 adjusted for age)

5.3.3 Level of MRWA

Participants who reported any MRWA were asked in the survey if they had pursued a variety of different types of MRWA and these are ranked by the percentages of people undertaking them (Table 5.5). Walking on the beach (87.6%) was the most widespread activity while windsurfing was the least (0.4%).

Recreational water users were then divided into three categories based on an estimation of the amount of exposure each activity would involve (Table 5.6). Participants who reported more than one type of activity were classified according to the highest level of exposure they reported. Questions were also asked of respondents on how long they were in the water on the day they had reported symptoms. Options of less than 30 minutes, 30 to 60 minutes and over 60 minutes were given.

Table 5.5 The proportion of the study population that undertook MRWA by specific activity.

	n	%
walking	936	87.6
wading	654	61.2
swimming	553	51.7
fishing (boat)	465	43.5
fishing (shore)	383	35.8
fishing (surf)	211	19.7
fishing (jetty)	160	15.0
surfing	138	12.9
castnetting	70	6.5
other	55	10.3
sailing	41	3.8
snorkeling/diving	36	3.4
skiing	27	2.5
crabbing	22	2.1
jetskiing	20	1.9
windsurfing	4	0.4

Table 5.6 Exposure levels of MRWA.

low	moderate	high
walking on beach	skiing	swimming
wading	jet ski	snorkelling/diving
sailing	windsurf	surfing
fish jetty	crabbing	
fish shore	castnetting	
fish boat	fish surf	

Table 5.7 Proportion of study population with different levels of water exposure by age, sex and smoking status.

		Level of water exposure							
	group	**none**		**low**		**medium**		**high**	
		no.	**%**	**no.**	**%**	**no.**	**%**	**no.**	**%**
age group (years)	**18-29**	8	7.5	12	11.3	7	6.6	79	74.5
	30-44	29	12.7	30	13.2	21	9.2	148	64.9
	45-59	101	22.1	109	23.9	62	13.6	184	40.4
	60-74	165	34.2	120	24.8	58	12.0	140	29.0
	75-90	55	67.1	15	18.3	2	2.4	10	12.2
sex	**male**	176	24.8	126	17.7	116	16.3	292	41.1
	female	191	29.2	159	24.3	33	5.1	270	41.3
current smoking	**yes**	28	14.9	31	16.5	25	13.3	104	55.3
	no	311	28.7	240	22.2	113	10.4	523	38.7

As degree of exposure to marine waters increased from none to high, the age of persons in each group decreased. Averages of ages decreased from 64.4 years for respondents not reporting MRWA, to 60.1, 58.1 and 50.9 for low, medium and high exposure groups, respectively. The average ages of exposure groups were significantly different apart from those in the low and medium exposure groups (ANOVA, $p < 0.05$). The age groups differed in the exposure level ($\chi^2 = 0.000$) (Table 5.7). The trend was for a higher proportion of the younger age groups to undertake high water exposure. The youngest age group (18-29 years) had the greatest high level exposure (74.5%) while the oldest (75-90 years) had the lowest (12.2%).

Males and females differed in their patterns of water exposure (χ^2, $p = 0.000$) (Table 5.7). A greater proportion of males than females had moderate exposure (16.3% verses 5.1%), and this is probably due to the types of activity allocated to the moderate exposure group. Significantly more men than women undertook medium

exposure activities such as water-skiing (χ^2, p=0.004), cast netting (χ^2, p=0.000) and fishing in the surf (χ^2, p = 0.000).

5.3.4 Symptoms

Participants who reported MRWA were asked if they had suffered from any of seventeen symptoms after contact with Moreton Bay waters (Table 5.8). Choices of none, mild, moderate or severe were given for each symptom. Of the 1007 people who engaged in MRWA, 349 (34.6%) reported at least one symptom over the seven-month period. Of those engaging in MRWA, the symptom most reported was skin itching (22.7%) while fever was the least (0.4%) (Table 5.8).

The average age of those reporting symptoms was 50.3 years (n = 348), compared to 59.6 years (n = 1007) for those not reporting symptoms (t test, p = 0.000). Age groups differed in the proportion of symptoms reported (χ^2, p = 0.000). The trend was for older age groups to have fewer symptoms. The percentage fell from 60.2% of the youngest group (18-29 years) having symptoms, while only 32.3% of the oldest age group (75-90 years) reported symptoms (Table 5.9). The percentage of males and females reporting symptoms did not differ (χ^2, p = 0.473). Proportionally more smokers reported symptoms than non-smokers (36.2% verses 23.7%, χ^2, p = 0.000) however this difference was no longer statistically significant after adjusting for age (Mantel-Haenszel summary χ^2 was p = 0.09).

In an attempt to simplify the data, groups of symptoms were combined under four headings, skin, gastrointestinal, eye and fever and headache symptoms (Table 5.10). Symptoms ranked from skin symptoms at the highest (26.9%) and gastrointestinal symptoms being the lowest (3.5%) (Table 5.11). Severe skin symptoms, the type that could be expected from high exposure to toxic *L. majuscula*,

affected 2.7% of those undertaking MRWA. If persons with wheals, which are often associated with cnidarian stinging episodes but not exposure to toxic *L. majuscula*, are excluded the percentage falls to 1.3%.

Table 5.8 The proportion of the study population that undertook MRWA who reported each symptom.

symptom	n	%
skin itching	229	22.7
sore eyes	168	16.7
skin redness	106	10.5
sore ears	68	6.8
skin burning	63	6.3
headache	62	6.2
discharge from eyes	35	3.5
skin wheals	22	2.2
other (described)	21	2.1
mouth ulcers	17	1.7
nausea	17	1.7
discharge from ears	15	1.5
abdominal pain	14	1.4
diarrhoea	14	1.4
skin blistering	14	1.4
skin swelling	8	0.8
vomiting	5	0.5
fever	4	0.4
any symptom	349	34.6

Table 5.9 Proportion of the study population reporting symptoms by age, sex and smoking status.

		reporting any symptoms		no symptoms			
		no.	(%)	no.	(%)	test	p
age group (years)	**18-29**	62	60.2	41	39.8	χ^2	0.000
	30-44	87	40.5	128	59.5		
	45-59	101	26.7	277	73.3		
	60-74	87	25.7	251	74.3		
	75-90	10	32.3	21	67.7		
sex	**male**	174	30.6	395	69.4	χ^2	0.473
	female	173	35.0	321	65.0		
current smoking	**no**	257	31.0	571	69.0	χ^2	0.000
	yes	68	41.0	98	59.0		

Table 5.10 Groupings of symptoms.

skin	gastrointestinal	eye	fever & headache
itching	abdominal pain	sore eyes	fever
redness	nausea	discharge from eyes	headache
burning	diarrhoea		
blistering	vomiting		
swelling			
wheals			

Table 5.11 Proportion of study population engaging in MRWA who reported symptoms in each group.

	n	%
skin	267	25.0
gastrointestinal	34	3.2
eye	181	16.9
fever & headache	65	6.1

A number of individuals reported symptoms of exposure to aerosolised *L. majuscula* toxins, with 245 of 1373 (17.8%) reporting breathing problems during the time-period of the study (Table 5.12). The majority of people did not have respirational problems during a bloom period.

Table 5.12 Potential symptoms of aerosolised toxic *L. majuscula* in Bribie Island population.

	never	%	once	%	sometimes	%	frequently	%
productive cough	508	74.9	17	2.5	110	16.2	43	6.3
dry cough	503	69.8	8	1.1	168	23.3	42	5.8
runny nose	420	56.1	19	2.5	214	28.6	95	12.7
sore throat	459	63.7	35	4.9	193	26.8	34	4.7
blocked sinuses	458	65.7	21	3.0	109	15.6	109	15.6
repeated sneezing	436	57.7	16	2.1	193	25.5	111	14.7
chest tightness	520	74.6	17	2.4	107	15.4	53	7.6
wheeze	533	76.9	13	1.9	99	14.3	48	6.9
sore eyes	458	59.5	27	3.5	205	26.6	80	10.4
discharge from eyes	569	84.4	18	2.7	62	9.2	25	3.7

No differences were found between proportions of males (30.6%) and females (35.0%) reporting symptoms overall (χ^2, $p = 0.473$) but greater numbers of women reported one or more skin symptoms (χ^2, $p < 0.05$) and fever and headache symptoms (χ^2, $p < 0.05$) (Table 5.13). Of the symptoms making up skin symptoms, only the proportion reporting skin itching differed significantly between men and women (χ^2, $p = 0.000$, data not shown).

Among those undertaking MRWA, significantly higher proportions of the younger age groups reported symptoms. This was found for skin, gastrointestinal, eye, and fever and headache symptoms (χ^2, $p = 0.000$ trend across all four symptom groups) (Tables 5.13). High exposure groups had a greater proportion of skin (χ^2, $p = 0.000$), gastrointestinal (χ^2, $p = 0.014$) and eye symptoms (χ^2, $p = 0.000$) symptoms (Table 5.13). No association was found between time spent in water and symptoms (data not shown).

Table 5.13 Proportion of the study population reporting types of symptoms by age, sex and exposure status.

	Skin		gastrointestinal		eye		fever and headache	
age group (years)	**no.**	**(%)**	**no.**	**(%)**	**no.**	**(%)**	**no.**	**(%)**
18-29	32	43.8	5	6.8	23	31.5	13	17.8
30-44	62	32.5	12	6.3	43	22.5	17	8.9
45-59	76	23.0	6	1.8	50	15.1	17	5.1
60-74	81	19.9	11	2.7	56	13.7	16	3.9
75-90	15	24.2	0	0.0	8	12.9	1	1.6
male	126	22.1	17	3.0	96	16.9	24	4.2
female	141	28.5	16	3.2	85	17.2	40	8.1
low exposure	44	15.3	3	1.0	20	7.7	12	4.2
moderate exposure	32	21.2	4	2.6	21	14.2	10	6.6
high exposure	180	32.0	27	4.8	135	24.0	42	7.5
*χ^2	12.28		3.14		21.32		0.000	
p	0.000		0.076		0.000		0.962	

*Mantel-Haenszel χ^2 for difference between high, and moderate and low water exposure by symptom adjusted for age

5.3.5 Association between Symptoms and Exposure

Logistic regression was used to investigate the relationship between level of water exposure, age, sex and reporting of symptoms. Separate models were constructed to look at all symptoms total symptoms, as well as skin, gastrointestinal, eye and fever and headache symptoms (Table 5.14). The crude association between level of water exposure and having symptoms is reported in Model I (Table 5.14). As level of water exposure increased, the odds of reporting any symptom compared to those with no water exposure was estimated to increase from 1.8 times at low exposure to more than 3.4 times at high exposure. This trend was repeated for skin, gastrointestinal and eye symptoms across the three exposure levels ($p < 0.05$). A similar trend was seen for fever and headache symptoms with water exposure across the three exposure levels although this did not reach statistical significance ($p = 0.066$).

The effect of degree of exposure on having symptoms remained strong after adjusting for age and sex. Models II and III show the effects of level of water exposure adjusted for characteristics of age (divided into 3 groups) and age and sex, respectively. Adjusting for age and sex decreased the -2 log likelihood value, suggesting improved model fit (from 1191.03 for Model I to 1172.11 and 1159.77 for Models II and III, respectively in the case of total symptoms). Addition of the age predictor variable attenuated the effect of exposure slightly, but this remained statistically significant. The addition of the sex predictor variable in Model III again attenuated the effect of exposure slightly for total, skin and eye symptoms (Table 5.14). The effect of exposure level on gastrointestinal symptoms no longer remains significant, probably due to the low sample numbers in the mild and moderate gastrointestinal symptoms (3 and 4, respectively).

Age was examined as older respondents were found to have lower exposure to water (Table 5.4 and Table 5.7), while younger people have more sensitive skin (Wilhelm, 1995). Model IV considers the association between age and symptoms. The odds of older respondents reporting symptoms was significantly less than younger respondents, with middle-aged (40-64 years) being 3 to 4 times less likely to report symptoms while older-aged (65-89 years) respondents were 4 to 5 times less likely to report symptoms than those aged less than 40 years. This trend was seen for combined symptoms, and all other symptom groups across three age groups ($p < 0.05$). When corrected for exposure level in Model V, all, skin, eye and fever and headache symptoms remain significantly different.

Sex of participant has also been found as a factor influencing not only type of water exposure, but also types and severity of symptoms (Table 5.7 and Table 5.13). Although the odds of female respondents reporting skin, and fever and headache symptoms was significantly higher than males, sex of respondents was not associated with reporting of gastrointestinal, or eye or combined symptoms (Model VI). After adjusting for level of exposure the association between gender and skin symptoms becomes stronger, with significantly more odds of reporting skin symptoms in females than males (Model VII). The effect of gender on fever and headache symptoms is no longer seen.

Table 5.14 Factors associated with symptoms: logistic regression.

		Odds Ratio (95% Confidence Interval)				
Co variable		**all symptoms**	**skin**	**gastrointestinal**	**eye**	**fever and headache**
exposure level						
Model I	**low**	1.0	1.0	1.0	1.0	1.0
(unadjusted)	**moderate**	1.8 (1.1, 2.9)	1.5 (0.9, 2.5)	2.6 (0.58, 11.9)	2.2 (1.2, 4.2)	1.7 (0.7, 3.9)
	high	3.4 (2.4, 4.9)	2.6 (1.8, 3.8)	4.8 (1.5, 16.1)	4.2 (2.5, 6.8)	1.9 (1.0, 3.6)
	p value for test of trend	0.000	0.000	0.006	0.000	0.066
age						
Model II	**younger (18-39)**	1.0	1.0	1.0	1.0	1.0
(unadjusted)	**middle-aged (40-64)**	0.3 (0.2, 0.5)	0.4 (0.3, 0.5)	0.2 (0.1, 0.4)	0.3 (0.2, 0.5)	0.3 (0.2, 0.5)
	older (65-89)	0.2 (0.1, 0.3)	0.3 (0.2, 0.4)	0.2 (0.1, 0.5)	0.3 (0.2, 0.4)	0.2 (0.1, 0.4)
	p value for test of trend	0.000	0.000	0.001	0.000	0.000
sex						
Model III	**(female verses male)**	1.1 (0.9, 1.4)	1.4 (1.0, 1.7)	1.0 (0.5, 2.0)	1.0 (0.7, 1.3)	1.8 (1.1, 3.0)
(unadjusted)						
	p	0.056	0.026	0.836	0.898	0.010
exposure level						
Model IV	**low**	1.0	1.0	1.0	1.0	1.0
(adjusted for age:	**moderate**	1.8 (1.1, 2.9)	1.5 (0.9, 2.5)	2.5 (0.6, 11.4)	2.1 (1.1, 4.1)	1.6 (0.7, 3.7)
in 3 groups)	**high**	3.0 (2.1, 4.2)	2.3 (1.5, 3.3)	4.0 (1.2, 13.6)	3.6 (2.2, 6.0)	1.4 (0.7, 2.8)
	p value for test of trend	0.000	0.000	0.019	0.000	0.375
exposure level						
Model V	**low**	1.0	1.0	1.0	1.0	1.0
(adjusted for age	**moderate**	1.9 (1.2, 3.1)	1.7 (1.0, 2.9)	1.9 (0.4, 9.7)	2.2 (1.1, 4.3)	2.0 (0.8, 5.0)
and sex)	**high**	3.0 (2.1, 4.3)	2.3 (1.6, 3.4)	3.9 (1.2, 13.4)	3.6 (2.2, 6.0)	1.6 (0.8, 3.1)
	p value for test of trend	0.000	0.000	0.180	0.000	0.288

5.3.6 Other Measures of Symptom Severity

As respondents to the questionnaire were asked to self-describe the severity of symptoms they reported, inter-individual differences may have occurred. In an attempt to better quantify severity of symptoms, others measures were utilised. One measure of this was whether those who had symptoms had attended health practitioners. Of respondents with symptoms that occurred after water contact, 15.0% attended health professionals. Pharmacists were attended the most, then GPs, specialists and least attended were hospitals. This tend occurred across all symptom groups. A smaller proportion of individuals with skin complaints attended health practitioners than gastrointestinal complaints (Table 5.16).

Table 5.16 Percentage of respondents with symptoms that attended health professionals.

	skin (n=267)		gastrointestinal (n=34)		eye (n=181)		fever & headache (n=65)	
	no.	%	no.	%	no.	%	no.	%
any health professionals	53	19.9	20	58.8	40	22.1	21	32.3
GP	23	8.6	8	23.5	15	8.3	10	15.4
pharmacist	26	9.7	11	32.4	22	12.2	10	15.4
hospital	1	0.4	0	0.0	0	0.0	0	0.0
specialist	3	1.1	1	2.9	3	1.7	1	1.5

To further assess severity of symptoms participants were asked how much money was spent on treatment. Twenty-seven respondents replied that they had spent money on treatment and amounts spent varied between $4.00 and $250.00, with an average of $12.60. Twenty-five of these respondents had had treatment for skin problems.

Fifty-one respondents replied they had problems sleeping because of symptoms acquired after exposure to Moreton Bay waters. An average of 25.0 nights sleep were affected, but a few individuals recording sleep problems lasting many

months skewed this average, with the range from one night to 365 nights (median 3.0 nights). Forty of these respondents with problems sleeping had skin complaints. Two persons took one day off work each, due to symptoms caused after water exposure (both had skin and eye symptoms).

Questions were asked regarding any changes of behaviour precipitated by symptoms. Of those that had symptoms, 92.9% said they would return to the same location that they incurred symptoms. Of these, 35.0% said they would change their behaviour next time they visited they site. Of those with any severe symptoms 76.7% would return to the same location but 34.9% would change their behaviour. Of those with severe skin symptoms, 75.9% would return to the same location but 65.5% would change their behaviour. Of those with sleep problems, 74.1% said they would return to the same location but 77.8% would alter their behaviour.

5.3.7 Knowledge and Attitudes towards *Lyngbya*

The vast majority of survey respondents had heard of *Lyngbya,* "fireweed" or stinging blue-green algae (87.7%). However, only 101 (7.3%) could correctly reply that *Lyngbya* caused skin, eye and breathing problems, but not ear or gastrointestinal symptoms (Table 5.17). The vast majority claimed they would actively avoid areas known to have *Lyngbya* (81.5%).

Table 5.17 Numbers of participants answering questions on *L. majuscula* symptoms correctly.

	number with correct answer (%)
skin rashes	1000 (73.0)
eye irritations	890 (65.0)
diarrhoea	729 (53.2)
breathing problems	566 (41.3)
earache	682 (49.8)
all questions	101 (7.3)

5.3.8 Pre-existing Atopy

Several studies have shown that individuals with pre-existing atopy are more likely to develop irritant contact dermatitis (Lammintausta and Kalimo, 1981; Baurle *et al.*, 1985; Nilsson *et al.*, 1985; Rystedt, 1985; Shmunes, 1986). In an attempt to eliminate this as a potential confounder, questions were asked on whether individuals had suffered from dermatitis, eczema, asthma or other allergies in the five years prior to the Bribie Island Survey (Table 5.18). Exclusion of individuals that reported suffering from eczema, dermatitis, asthma or allergies in the last five years reduced the numbers reporting severe skin symptoms from 2.7% to 2.5%.

Table 5.18 Percentages of individuals reporting potential atopy.

	n	% of those repying to survey	% reporting skin symptoms	p
eczema	91	6.6	35.2	0.000
dermatitis	171	12.5	31.0	0.000
asthma	197	14.4	28.4	0.001
allergies	279	20.4	25.1	0.010
any	487	35.5	25.5	0.000

5.3.9 Exposure to Sun

Excess exposure to sun may cause some symptoms similar to those of *L. majuscula.* We asked volunteers if they used sunscreen/sunsafe tops in an attempt to examine if these articles reduced episodes of skin complaints (Table 5.19). Wearing of sunscreen did not increase or decrease the likelihood of having a skin complaint (χ^2, $p < 0.05$). Wearing of sunsafe tops increased the likelihood of mild skin complaints only (χ^2, $p = 0.005$).

Table 5.19 Protection from the sun and reporting of skin symptoms.

	n	% of those replying to survey	% reporting skin symptoms	p
sunscreen	881	82.4	25.0	0.408
sunsafe	330	30.9	29.4	0.026

5.3.10 Monitoring Bacterial Pathogens in North Deception Bay

Bacteriological assessments by the Environmental Protection Agency, Queensland Government of concentrations of faecal coliform (thermo-tolerant coliform) bacteria were used to examine the microbiological water quality in the UQ Health of Ocean Users survey area (Webb, 2001, 2003). Presence of these organisms in high numbers would indicate that the water was contaminated with faecal material, which may contain harmful bacteria, viruses and parasites. Australian and New Zealand Environment and Conservation Council (ANZECC) water quality guidelines were used to assess the suitability of sites within the UQ survey area for primary and secondary contact recreation (ANZECC, 1992; 2000). Primary contact is considered to be activity with direct exposure to water such as swimming, skin diving, water skiing, and similar, where there is a high probability of water being swallowed. It usually involves human body contact with water where such activities take place in an organized manner, or on a frequent basis. Secondary contact includes such activities as sailing, canoeing and fishing, for which there is a lower risk of swallowing water (Webb, 2003).

Sites examined by the EPA that fell within the study area included Banksia Beach, Sylvan Beach, Bongaree and Woorim Beach. All four sites complied with ANZEEC guidelines for primary contact recreation, and hence secondary contact recreation during the periods October-November 1998, January-February 1999, March-April 1999, March-April 2000 (Webb, 2001). October-November 2000, January-February 2001 and March-April 2001 complied 100% with ANZECC water quality guidelines for primary water contact (Webb, 2003).

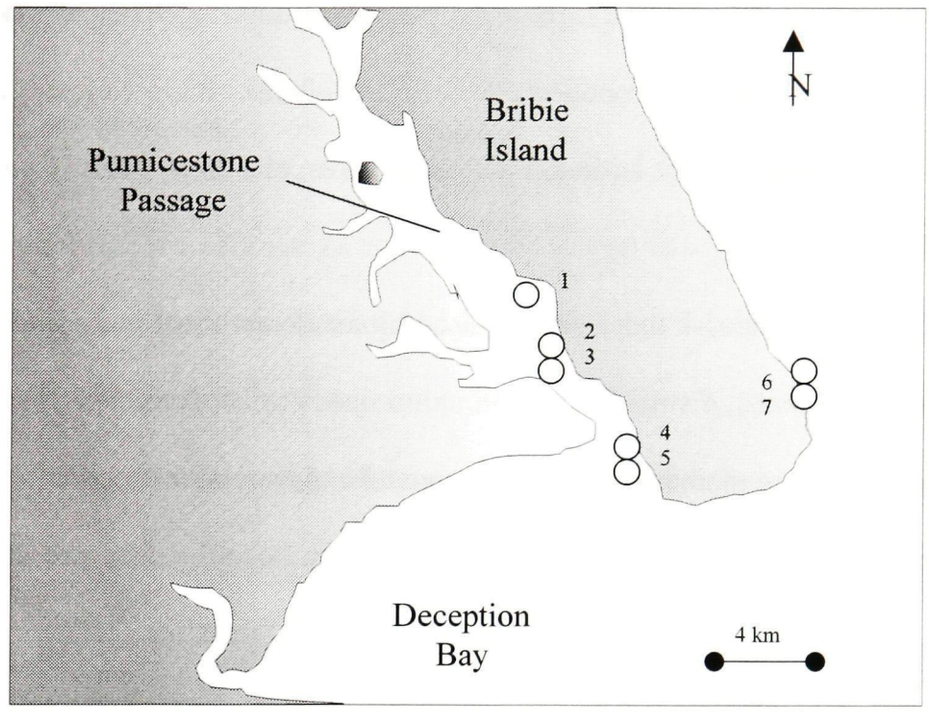


Figure 5.4 Sampling sites for EPA water quality summer 2001-2002 (1, Sylvan Beach; 2, Banksia Beach; 3, Banksia plume; 4, Bongaree; 5, Bongaree plume; 6, Woorim; 7, Woorim plume).

5.4 Discussion - Bribie Island Study

5.4.1 Introduction

Many factors increase the chance of morbidity and mortality of occupational and recreational water users over those who choose to stay on dry land. These include the water quality, the presence of harmful blooms of dinoflagellates or cyanobacteria, pathogenic organisms, anthropogenic and natural chemicals, as well as the risks and hazards of the activity that an individual undertakes, and the types of equipment they use.

To assess the risk that may be posed by an element that causes adverse health outcomes in humans, a variety of information needs to be examined. Epidemiology, toxicology, clinical medicine and environmental exposure assessment all contribute to a process resulting in a conclusion whether a health hazard exists, and an estimation of the magnitude of associated risk (World Health Organisation, 2000). As the main focus of the 'Health of Ocean Users' survey was *L. majuscula*, most of the discussion will primarily centre on severe skin irritation as this is the main symptom associated with *L. majuscula.*

Five thousand postal surveys were delivered to the residents of Bribie Island and neighbouring mainland suburbs, and 1370 were returned (27.4%). The respondents approximated the population demographic living in this area as measured and described by the ABS. High numbers of individuals participated in marine recreational water activities (78.2%). Of those engaging in MRWA, 25.0% reported skin symptoms, the majority being mild, with only 2.7% reporting severe skin symptoms. Using logistic regression, it was found that both increased skin and eye symptoms were associated with an increased level of water exposure, when adjusted for age and sex. Younger age groups were more likely to report skin, eye, or fever

and headache symptoms, after adjusting for exposure level. The odds of women reporting skin symptoms were 50% higher than men, after adjusting for exposure level.

These results show that over a seven-month period, in an area that had been shown to have toxic blooms of the marine cyanobacterium *L. majuscula*, only small numbers of people reported skin symptoms comparable in severity to those shown in other locations where outbreaks of this toxic *L. majuscula* have been reported. This shows that the presence of toxic *L. majuscula* in waters where MRWA occurs does not necessarily mean large numbers of individuals will suffer severe skin symptoms. Young females appeared to have a greater chance of reporting skin symptoms than other groups. These results will be discussed more fully after threats to validity have been addressed.

5.4.2 Chance

It was thought that chance played little role in the results described in this study with $p < 0.05$ set as the level of significance. Of those replying to the survey, the average age of participants engaging in MRWA was less than those who were not. This difference is unlikely to be due to chance as a statistically significant trend across five age groups revealed younger respondents more likely to be engaging in MRWA. The level of exposure to marine waters was also associated with the age of a person. This difference is unikely to be due to chance as a statistically significant trend could be tracked across several age groups.

Increasing exposure from low to moderate to high saw an increased reporting of skin and eye symptoms. This difference was still present after adjusting for age and sex. The odds of individuals having skin and eye symptoms (2.3 and 4.2,

respectively) are large enough and the confidence intervals narrow enough to expect that these statistically significant differences are unlikely to be due to chance. The trend that greater the exposure the greater the reporting of symptoms is maintained with the odds at moderate exposure being lower than that at high exposure for both skin and eye symptoms. Reporting of skin, eye and fever and headache symptoms have been shown to be associated to the age of the participant. The younger age group (18-39 years) reported significantly more symptoms (OR = 2-2.5) than those from the middle-aged (40-64 years) and the older (65-89 years) age groups. These differences were retained once the model was adjusted for exposure level. This statistical difference does appear to be likely as it is a large difference in odds with narrow confidence intervals. There was however, no difference seen between middle-aged and older groups in their reporting of symptoms. Females reported more skin symptoms than males. It appears unlikely to be due to chance, as it is statistically significant and is maintained even when correcting for exposure level.

5.4.3 Selection Bias

The possibility of non-respondent bias is high, as the majority of individuals did not reply to this survey. Questions were asked of the participants so a comparison could be made with ABS surveys of the Bribie Island area that had a far greater response rate, to assess the strength of potential non-response bias. The strength of the Bribie Island Study was that the demographics of the participating population generally resembled that of the population of Bribie Island as recorded by the ABS in the 1996 *Census of Population and Housing* in relation to age and numbers in the labour force.

Results from this survey have shown a small but statistical difference between proportion of males and females residing on Bribie Island, as measured by the ABS, and the proportion replying to the Bribie Island Survey. It is plausible that the group replying to the survey differs from the group residing on Bribie Island, and has a higher proportion of males. A slightly higher proportion of males undertook MRWA but this was not statistically significant. This result may be due to a respondent bias, with more males than expected replying to the Bribie Island Survey. It is unclear how this may have affected the outcomes of the Bribie Island Study.

Large differences in the numbers of unemployed persons were detected between the Bribie Island Survey and the ABS statistics (4.0% verses 17.9%, respectively). The primary difference is that the ABS figure included people 15 years and above, where the Bribie Island Study included only those 18 years and older. High numbers of unemployed persons are traditionally in the younger age group. However, some of the difference in percentage of unemployed persons responding may be partly due to a respondent bias. Unemployed persons have also been shown to have an unwillingness to respond to population surveys and this is thought to be due to the non-motivation personality characteristics of this population (Novo *et al.*, 1999). This has also been shown by Korkeila *et al.* (2001), but the difference was not to such a great extent (OR, (95% CI) = 0.92 (0.88, 0.97) for unemployed replying to a survey). This may have led to a selection bias. It is unknown how this may have affected the Bribie Island Survey. While there is a large difference in the numbers of unemployed replying to the survey, number reporting not being in the labour force were very similar (63.9% verses 63.5% for Bribie Island Study and ABS, respectively).

A lower proportion of smokers than expected appeared to reply to the Bribie Island Survey. This is probably a real difference given the size of the difference (approximately 9% lower). This difference was measured against the national statistic, as no smoking information was available for Bribie Island, and hence does not take into account the potential of a different age distribution in the Bribe Island area surveyed.

Those in the population who received a survey form but did not have any health problems may not have responded in such great numbers as those that had symptoms after MRWA, especially those with severe symptoms. In an effort to reduce this, questions were initially asked on exposure rather than symptoms, in an attempt to gain participation from individuals who suffered no symptoms. Large numbers of participants did report no symptoms (n = 1021). But similarly, large numbers of the population did not reply at all (n = 3630). This may have lead to more people with symptoms responding.

Publicity bias may have occurred with the Bribie Island Survey. The presence and harmful effects of *L. majuscula* has been reported in the media extensively over the preceding few years (from 1998 onwards). An indication of the knowledge by the general public about this organism, and its health effects, is measured in the survey with 87.7% of participants having heard of it, and 73.0% knowing it caused skin rashes. In an attempt to limit this, the survey was titled the '*Health of Ocean Users Survey*', questions were asked on a variety of symptoms and exposures, and no publicity from the media sought to promote the survey. Publicity bias may have led to an over-reporting of severe skin symptoms. This would only have affected the association between symptoms and exposure if these persons also have greater exposure that the non-respondents.

5.4.4 Recall

A respondent's ability to recall an injury is influenced by many factors. The primary reason is the length of time between the injury and the interview/questionnaire but other factors such as characteristics of the respondent, circumstances in which the injury was incurred, and the nature and severity of the injury may also be important (Massey *et al.*, 1976; Carlsson, 1983; Landen *et al.*, 1995). Some of the effect of difficulty in recall will be similar across all groups (non-differential) and can be designated recall error. Some difficulty in recall will be different in different groups (differential) and hence may lead to recall bias.

Difficulty in recalling symptoms may lead to recall error and an underestimation of incidence of symptoms. This study examines a large time-period (seven months) and so potentially, recall error may have occurred. Recall error is thought to be primarily due to memory decay (Cash and Moss, 1972; Coughlin, 1990; Harel *et al.*, 1994). Mock *et al.* (1999) found that participants asked to recall injuries over long periods significantly underestimate injury rates compared to short periods. Inability to recall symptoms may have led to an underestimation of symptom numbers, but not necessarily affected the association of symptoms with level of water exposure.

Those with minor symptoms may not have recalled them and hence not agreed to participate in the survey, or report them if they did. Authors have found the greater the severity of injury, the better the recall (Massey *et al.*, 1976; Carlsson, 1983). Therefore, in general, there may be an under-reporting of symptoms in this study, especially in the reporting of mild symptoms, with the implication that the proportion of severe symptoms would appear to be higher. This would, however, not necessarily

affect the association between the level of exposure and symptoms, unless under-reporting varied with exposure level.

Younger participants in this survey may have remembered symptoms better than older respondents. Younger participants in the present study reported five times more symptoms than older respondents. This may be due in part to recall error, and therefore greater under-reporting of symptoms, amongst older participants, although it seems unlikely to explain all of this difference. Groups examining more serious or high morbidities have found that younger people may be able to recall injuries better. A 3% difference between 50 and 60 year olds was found in recall of injuries recorded in a hospital registry (Carlsson, 1983). Massey *et al.* (1976) found that those in the 17-24 age group recalled the highest percentage of injuries followed by the 65 and older group and then the 45-64 year old group. There may have been an under-reporting of symptoms by older participants, but this would probably not explain all the difference found. A more accurate reporting of severe symptoms relative to mild and moderate symptoms would lead to a distinct possibility of over-reporting of the proportion of severe symptoms. Again, this would not necessarily affect the association of symptoms with level of water exposure, unless it varied with level of MRWA.

The study was titled "Health of Ocean Users" and did not ask questions on symptoms until after the exposure section was completed, in an attempt reduce recall bias. While a study subject may formulate the hypothesis that certain exposures may affect the risk of negative health outcomes from *L. majuscula*, based on the questions in the study, it is unlikely that respondents altered their answers. Hence, the recall bias is not thought to have a major influence on this survey.

5.4.5 Potential Confounding - Factors Leading to Irritations in the Marine Environment

Potential confounders in this study include age, sex, sun, pre-existing atopy and other factors that may be present in water. Age and sex have been eliminated as confounders as they were controlled for using logistic regression models (Table 5.13 in results section).

As it has been found that there was an association between water exposure and skin and eye irritation, potential confounders need to be explored. Many factors in the marine environment do cause irritation in humans, apart from toxic *L. majuscula.* Risk factors to be considered include seawater itself, exposure to sun, pre-existing atopy, pollutants and exposure to other marine organisms. These organisms include microorganisms, enteric pathogens, cnidarians, sea lice, cercairae and other cyanobacteria and dinoflagellates. A vast array of natural microbiological dermatological hazards (Auerbach, 1987) and other more exotic causes of dermatoses (Mandojana and Sims, 1987) are associated with the aquatic environment.

Some of these factors are present at Bribie Island. Attempts were made during the survey to eliminate potential confounders by obtaining environmental data or asking particular questions on the behaviour or health status of individuals. Again, it is emphasised that the severe skin or eye irritation accompanying exposure to toxic *L. majuscula* is the key interest of the present study.

5.4.6 Exposure to Water

Exposure to seawater or elements in it may have led to confounding. Seawater itself has been found to have both irritant (Agner and Serup, 1993; Ramsing and Agner, 1997) and anti-inflammatory properties (Scholtz, 1965). It has not been

recorded to cause severe irritation symptoms. Recent work by Yoshizawa *et al.* measuring transepidermal water loss has confirmed that seawater, sodium chloride solution, potassium chloride solution, and to a lesser extent magnesium chloride solutions reduces irritation by the classic experimental irritant sodium lauryl sulphate. The authors concluded that this reduction in irritation could be attributed to skin barrier preservation by sodium chloride solution and potassium chloride solution, and an emollient effect by sodium chloride solution (Yoshizawa *et al.*, 2001). Recent epidemiological studies have reported the benefits of bathing of in saltwater in communities with chronic skin and ear disease (Lehmann *et al.*, 2003). While mild transient itching after exposure to water is common, severe pruritis (such as aquagenic pruritis, polycythemia rubra vera and aquagenic pruritis of the elderly) is uncommon (Steinman, 1987). This evidence suggests that while seawater may lead to increased reporting of mild skin itching, bathing in seawater does not commonly induce severe skin symptoms. This will therefore not lead to a higher proportion of severe skin symptoms being reported by those who have greater contact with seawater.

Pollutants in recreational bathing waters are a potential cause of irritation. For example, populations living close to wood processing plants have previously been found to have higher rates of skin symptoms over control populations (Dahlgren *et al.*, 2003a; Dahlgren *et al.*, 2003b). Measurement of pollutants is regularly undertaken by the Queensland Government and must fall within guidelines agreed to by the Queensland Government and legislated in the Environmental Protection (Water) Policy, 1997 (Queensland Government, 1997), in combination with the Environmental Protection Act, 1994 (Queensland Government, 1994). Major industries in the area include forestry (39% of land-use of catchment) and horticulture (9%). Over 50 "environmentally relevant activities" including chemical processing,

wood production/treatment and aquaculture occur in the catchment area. Urban areas only account for 4.4% of land use in this catchment (South East Queensland Regional Water Quality Management Strategy, 2001). Close monitoring of water quality by the Queensland Government is thought to eliminate this as a potential cause of severe skin symptoms, and therefore pollutants are unlikely to have led to an over-reporting of severe skin symptoms.

5.4.7 Exposure to Marine Organisms

Many organisms as well as the physical dangers make MRWA hazardous. A variety of authors have cited many of the dangerous aquatic organisms in marine recreational waters, including species of fish, reptiles, mammals, cnidarians, molluscs, annelids and echinoderms (Manowitz *et al.*, 1979; World Health Organisation, 1990; World Health Organisation, 2003). Many of these produce symptoms similar to exposure to toxic *L. majuscula.* Contact with these organisms may lead to the over-reporting of *Lyngbya*-like symptoms. Some of these organisms are present in the Bribie Island area, although they may not be very common.

A vast array of microbes has been found to exist in the marine environment including bacteria, microalgae, protozoa, fungi, yeast and viruses. Irritation and dermatitis may be caused by some of these species (Collier, 2002). *Erysipelothrix rhusopathiae* is a facultative aerobic inciting bacillus (rod) agent of infection known as "speck finger" or "fish-handlers disease". Vibrio species may also cause infection via skin wounds or ingestion of contaminated seafood. *Vibrio vulnificus*, a species particularly virulent when invading wounds, has been found to favour warmer water (>20°C) with low salinity, as may occur in sub-tropical estuarine areas (Oliver *et al.*, 1983), that would include Pumicestone Passage. Infective *Vibrio* species have been

found in samples of *L. majuscula* (Sims *et al.*, 1993). *Mycobacterium marinum* has also been implicated in cutaneous infections (Kullavanijaya *et al.*, 1993). It should be noted that infections with marine bacteria are rare. Patients with leg ulcers, peripheral vascular disease, diabetes, or receiving immunosuppressive drug treatment may acquire unusual infections after saltwater exposure (Lambertus *et al.*, 1988; Papanaoum *et al.*, 1998), but these can be seen as rare cases and would lead to minimal over-reporting of symptoms.

Enteric pathogens enter the marine environment from terrestrial ecosystems and have been found to induce a variety of symptoms including gastrointestinal, respiratory, dermatologic, and ear, nose and throat infections. Many studies have examined urban runoff and increased incidences of a variety of symptoms have been found amongst swimmers verses non-swimmers at polluted beaches (De Donno *et al.*, 1994; Ferley *et al.*, 1989; Fleisher *et al.*, 1998; von Schirnding *et al.*, 1992). Two factors are present which allude to the conclusion that over-reporting of severe skin symptoms did not occur due to exposure to enteric organisms. Firstly, the area examined by this study was found to not have enteric bacteria above the ANZEEC guidelines during the period of the study, and for years before and after it. Secondly, gastrointestinal symptoms were lower in frequency than skin symptoms, the reverse of what has been found in surveys of recreational water users bathing in waters shown to contain enteric bacteria. Only 3.5% of participants of the Bribie Island Survey reported gastrointestinal symptoms after water exposure. If enteric bacteria were present, it would not be a major contributor to skin and/or eye irritation, and even less so to severe irritation.

Exposure to Cnidarians or their stinging parts, often broken up in the surf and made invisible to the naked eye, can cause a variety of skin irritations (Aguilera,

1973; Auerbach 1987; Auerbach and Hays, 1987; Burnett *et al.*, 1987; Halstead, 1987; Frenk *et al.*, 1990; Kokelj *et al.*, 1989; Kokelj *et al.*, 1993; Burke, 2002a). Several species of "marine stingers" have been noted in Moreton Bay waters including *Physalia*, *Catostylus*, *Cyanea*, *Tamoya*, *Pelegia* (Davie, 1998). The common name of *Tamoya* is in fact "Morteon Bay stinger". "Seabathers eruption", a highly pruritic eruption under swimwear that occurs after bathing in the ocean (Sams, 1949; Macsween and Williams, 1996), has been found to have cnidarian larvae as its causative organism (Freudenthal, 1991; Freudenthal and Joseph, 1993) mimicking exposure to toxic *L. majuscula* in that it involves irritation under bathing costumes, especially the inguinal region. In the data collected by Freudenthal (1993), pruritis was severe in 68.0% of 70 cases, with 98.6% of cases reporting some level of pruritis. Lesions were described only on covered areas in 68% of cases. The remainder involved both covered and exposed areas. Fatigue/malaise and fever were found in 23.0% and 18.6% of patients with sea bather's eruption (Wong *et al.*, 1994). Wheals can be expected from exposure to cnidarian tentacles and in this study these were found in only a small number of cases reporting skin complaints. This suggests that the majority of cases of severe irritation were due to something other than exposure to cnidarians and that over reporting of skin symptoms due to cnidarians, if it did occur, was only a minor component.

Small marine crustaceans of the order Isopoda, suborder Cymothoidae, cause sea louse dermatitis. These organisms frequent sandy bottoms below the water level and feed on higher marine mammals, but also attack humans (Best *et al.*, 1964). The bite is rapid and sharp and causes a haemorrhagic puncture wound (Fisher, 1995; Burke, 2002b). These members of this suborder have not been recorded in Moreton Bay but have been reported in Queensland waters (Bruce, 1987). These symptoms are

not like those described in the majority of cases of those describing skin complaints. Of those reporting itching, redness, burning or a combination of these, only very few also reported wheals (3.0%) and swelling (1.5%), responses expected from puncture wounds. Hence, over reporting of severe *Lyngbya*-like skin symptoms due to exposure to sea lice is thought to be unlikely.

Similarly, exposure to cercariae has been linked to rash, with a punchate appearance progressing to erythema (Appleton *et al.*, 1979; Gonzalez, 1989). Wheals are common among individuals reporting contact with organisms (81.5% of those who had rashes) (Anon., 88/89). In the present study, only 8.6% of those that reported skin complaints had wheals. If the majority of these instances of skin complaints were due to schistosome cercariae then we may expect to see a higher occurrence of wheals in sufferers of skin rash.

There are more than 5000 species of marine microalgae from five major divisions, *Chlorophyta*, *Chrysophyta*, *Pyrrhophyta*, *Euglenophyta* as well as *Cyanophyta*, and of these some 200 have exhibited toxicity (Landsberg, 2002). Some of these 200 species do exist in Bribie Island waters and could be contributing to the irritancy found in this area. These may lead to an overestimation in the number of cases on skin or eye irritation.

The marine cyanobacteria of the genus *Trichodesmium* have been found to contain paralytic shellfish toxins (Jackson *et al.*, 2001) and to cause respiratory and dermal irritation (Sato *et al.*, 1966). This genus is known to occur in blooms in Queensland waters (O'Neil, 1998). The chance of over reporting due to the presence of other harmful algal blooms is slim as no reports of these blooms were made during the period of the questionnaire and blooms of these other algal species are rare in this area.

5.4.8 Exposure to Sun

The wording of the question "did you suffer any of the following, "*after contact with the water*" (underline and italic present in survey) was intended to indicate that only symptoms associated with exposure to water were of interest, and hopefully led to the elimination of reporting of skin complaints due to sunburn (itching, burning, redness, swelling, blistering). Wearing of sun safetops may decrease the incidence of sunburn, but wearing of clothing in the water has been found to increase both incidence of skin complaints caused by both *L. majuscula* (Grauer *et al.*, 1961) and cnidarians (Wong *et al.*, 1994). These would however be expected to be severe skin complaints. Wearing of these tops may lead to slight abrasions leading to mild skin complaints. As wearing sunscreen was not associated with the incidence of skin symptoms and wearing of sunsafe tops increased the incidence of mild skin symptoms alone, over-reporting of severe skin symptoms due to the inclusion of sunburn was thought to be minimal. As wearing sunscreen and sunsafe tops was associated with level of MRWA, sunburn could increase not only the reporting of symptoms, but also the association between water exposure and symptoms.

5.4.9 Pre-existing Atopy

Several studies have demonstrated higher risk for atopic persons to develop irritant contact dermatitis (Baurle *et al.*, 1985; Lammintausta and Kalimo, 1981; Nilsson *et al.*, 1985; Rystedt, 1985; Shmunes, 1986). Individuals not only have a lower threshold for irritation but also slower healing (Wilhelm *et al.*, 1990). Exclusion of individuals with pre-existing atopy led to only a small reduction in the number of individuals reporting severe skin symptoms (2.7% to 2.5% of those

undertaking MRWA). This difference suggests pre-existing atopy may have led to a slight but negligible over-reporting of severe skin symptoms. Atopy may also confound the association between the level of water exposure and symptoms as it was found to be related to level of exposure.

5.4.10 Conclusions - Factors potentially affecting results

The results of the Bribie Island Study do stand up once chance, bias and confounding have been considered. Chance has shown to be an unlikely factor as results were statistically significant ($p < 0.05$). Bias was present but did not fully explain all results seen. It is likely that there is recall error but it is unlikely to explain the extent of the association seen. Again, the association persisted after adjusting for confounding. It can be concluded that the associations of skin and eye symptoms, but not gastrointestinal or fever and headache symptoms, with level of water exposure is real. Other factors such as age, sex were controlled for in the models, and others such as sun, pre-existing atopy, and other potential irritants in the water have generally been excluded and not thought to explain all the results seen. Hence, increasing exposure to water during a season when toxic *L. majuscula* was know to be present in the Bribie Island area is associated with increased reporting of symptoms similar to those ascribed to *L. majuscula.*

5.4.11 Results in Context Based on Previous Work

This is the first observational study of the effect of a marine cyanobacterium on the health of a population. Previously, only retrospective analysis of data from individuals or small groups that had been exposed to toxic *L. majuscula* has been undertaken (Grauer *et al.*, 1961; Izumi *et al.*, 1987; Anderson *et al.*, 1988).

With a response rate of 24.9% the survey response rate is somewhat lower than other work undertaken using postal surveys (35-49.9%) (Harrison *et al.*, 2002; Kadyk *et al.*, 2003). The low response rate of this survey is probably a reflection of the importance of the issue in the population, and is itself a measure of the population's health concerns. Philipp (1992) and Philip *et al.* (1992) achieved a response rate of 43% and 68%, respectively, in his postal survey on exposure to freshwater cyanobacteria. These higher rates were probably due to the survey target population being members of the recreational clubs who were previously impacted by exposure to cyanobacteria. In an effort to increase response rates a reminder postcard was used and this technique elicited 176 requests for a new survey form as well as an increase in respondents using the original survey form. The decision was taken to use a reminder postcard in preference to other forms of incentive (such as financial) as other authors have found that reminder postcards are the most effective way of gaining higher response rates (Asch *et al.*, 1997).

5.4.12 Level of Exposure

An association was found between the level of water exposure and reporting of symptoms. Those with higher exposures were more likely to report symptoms, particularly skin and eye symptom. Previously it has been reported that exposure to *L. majuscula* leads to symptoms involving skin and eye symptoms (Grauer *et al.*, 1961; Solomon *et al.*, 1978; Izumi *et al.*, 1987; Anderson *et al.*, 1988). The association remained after adjusting for age and sex.

Increasing level and time of exposure would theoretically increase the chance of continued exposure to *L. majuscula.* This survey has shown increased level of exposure, but not duration of exposure, during MRWA increases the reporting of a

variety of symptoms including those similar to *Lyngbya*-like symptoms. A potential reason for the duration of exposure not being associated with reporting of symptoms may be the fast acting nature of the toxins associated with *L. majuscula.* This has been demonstrated both anecdotally and experimentally in several cases (Grauer *et al.*, 1961; Solomon *et al.*, 1978). Similarly, the injuries inflicted by exposure to cnidarians or their stinging parts have a short time period from exposure to symptoms appearing (minutes). Conversely, exposure to bacteria has an effect that is measured over a longer time-period (time scale of infection rather than intoxication). Hence, duration of exposure did not show an association with *L. majuscula.*

5.4.13 Symptoms

Many participants reported symptoms after bathing in Moreton Bay waters (34.6%). Skin itching (22.7%) was the symptom most commonly reported after MRWA with all skin symptoms reported by 26.9% of the population engaging in MRWA.

It is difficult to draw conclusions from other studies examining exposure to *L. majuscula.* These studies have primarily reported symptoms after a single exposure to what was presumed to be *L. majuscula* (Grauer *et al.*, 1961; Serdula *et al.*, 1982; Izumi *et al.*, 1987). These reports noted high numbers of individuals reporting symptoms on a single day, at a single site. In the present study, reports were from multiple sites over a seven-month period. An outbreak in 1980 in Hawaii saw 44.7% of those exposed attending a physician (Serdula *et al.*, 1982). Similarly 242 swimmers of 274 attending a beach picnic on Okinawa reported acute dermatitis. It appears that if *L. majuscula* is present and toxic and in a form that creates opportunities for exposure (i.e. broken up in surf and onshore winds) then large

numbers of individuals may be affected. This does not appear to be occurring in the Bribie Island area (Table 5.12).

Severe skin symptoms, the type that could be expected from high exposure to toxic *L. majuscula*, affected 2.7% of those undertaking MRWA. If persons with wheals are excluded, as they are often associated with cnidarian stinging episodes but not exposure to toxic *L. majuscula*, the percentage falls to 1.3%. It can be concluded from this number that if severe skin symptoms were being caused by exposure to toxic *L. majuscula*, the majority of individuals undertaking MRWA were not exposed or not exposed to high enough levels of toxins of *L. majuscula* to cause symptoms.

5.4.14 Age

Younger aged individuals participated to a greater extent in MRWA in this study. This has also been found in studies of freshwater recreational activity where the most numerous numbers of responses were from the 20-29 year age group in the study by Pilotto *et al.* (1997) and the 20-35 years age group in the study by Philipp (1985). A slightly higher proportion of males took part in MRWA. Pilotto *et al.* (1997) also found a high proportion of males participating in recreational water activity. Not only did younger people undertake MRWA more than older people but also the types of activities they engaged in were also higher in exposure.

Older persons reported less combined symptoms, skin, eye, and fever and headache symptoms than younger persons. This remained true after adjusting for level of exposure. This result was not unexpected, as age differences in irritant contact dermatitis have been reported in the literature. Using acute irritation patch testing, Robinson (2002) found the oldest cluster of their subjects (56-74 years of age) was significantly less reactive that younger age clusters. These findings have also

been found in other research involving the irritants ammonium hydroxide, poison ivy and sodium lauryl sulphate (SLS) (Coenraads *et al.*, 1975; Grove *et al.*, 1982; Lejman *et al.*, 1984; Cua *et al.*, 1990) as well as epidemiological data (Malten *et al.*, 1971; Robinson, 1999). As a group, young people have also been found to have greater levels of allergic dermatoses due to the wearing of nickel jewellery and cosmetics (Nixon, 1996; DeLeo *et al.*, 2002).

A reduction of transepidermal water loss (TEWL) in response to irritation by SLS has been noted with age, and may partly explain the phenomenon of the decrease in susceptibility to skin irritation with age (Berardesca and Distante, 1994). This has been noted that although a reduction in TEWL and older persons having a stronger barrier and consequent poorer penetration as a single explanation is unlikely (Wilhelm and Maibach, 1990).

These findings are by no means conclusive as other authors have shown that the permeability barrier is not compromised in older people by a range of methodologies including TEWL, percutaneous penetration and skin irritation (Tagami, 1971/1972; Leveque, 1989; Roskov *et al.*, 1989; Cua *et al.*, 1990; Wilhelm *et al.*, 1993). Age differences in irritation to SLS differ depending on the anatomical site of the skin (visual erythema scores and TEWL) (Cua *et al.*, 1990; Wilhelm, 1995). Intra- and inter-individual variation in skin irritation responsiveness may explain some of the inconsistencies in the literature, but not all (Robinson, 2001).

5.4.15 Sex

The odds of females reporting skin symptoms was 1.5, after adjusting for exposure level (Model III, Table 5.13). This was primarily due to increased reporting of skin itching. Earlier reports had suggested *L. majuscula* affected neither men or

women to a greater extent. Serdula *et al.* (1982) reported 21 persons studied after exposure to *L. majuscula*; all seven men and eight of 14 women had a rash. The difference in sexes approached, but did not reach statistical significance (Fisher's exact test $p = 0.08$) (Serdula *et al.*, 1982).

Although it is a common belief that women have more delicate integument than men do (Wilhelm, 1995), most studies have not shown this (Bjornberg, 1975; Frosch *et al.*, 1980; Lammintausta *et al.*, 1987; Cua *et al.*, 1990; Reed *et al.*, 1995; Shenefelt, 1996). Small but significant differences in skin susceptibility to irritants have been found between early and middle points of the menstrual cycle (Agner *et al.*, 1991). Premenstrual exacerbation of allergic contact dermatitis has also been noted (Rohold *et al.*, 1994). Allergenic dermatitis has also been reported higher in women due to the wearing of nickel jewellery and cosmetics (Nixon, 1996; DeLeo *et al.*, 2002).

Some sex differences in irritant contact dermatitis have been found. Males have been found to be significantly more sensitive to a suite of four chemical irritants (Robinson, 2002). Epidemiological studies have shown greater female sensitivity, but these studies bring in differences in chemical exposure patterns, so introducing another variable, which may skew results (Malten *et al.*, 1971; Lantinga *et al.*, 1984).

A potential reason for greater female susceptibility to skin symptoms from *L. majuscula* may be swimming costumes. It has been found for both *L. majuscula* (Grauer *et al.*, 1961; Fisher, 1995) as well as cnidarians or their planular larvae (Wong *et al.*, 1994; Macsween and Williams, 1996), that a prolonged exposure is facilitated by swimming costumes trapping these organisms. This extends contact with the toxin(s) or venoms, as opposed to toxic or venomous organisms resting briefly on the skin before being washed away. The greater surface area of many female swimming

costumes increases the likelihood of these events occurring. Stings of cnidarians skin symptoms allegedly due to *L. majuscula* exposure have been reported in the upper abdomen areas in females but no reports exist for males.

An alternative possibility is that females react differently to males when exposed to the toxins of *L. majuscula.* A differential number of protein kinase C receptors between males and females, the mode of action of *L. majuscula* toxins LA and DAT, is a possibility (Lachowicz *et al.*, 2002; Thompson and Khalil, 2003). No differences have appeared in a report of respiratory irritation, presumed to act via the same receptor group as skin irritation. No statistically significant differences in susceptibility to symptoms based on sex were found in 105 individuals who inhaled aerosolised *L. majuscula* toxin(s) (Anderson *et al.*, 1988). This does not rule out the possibility that there are a differential distribution of protein kinase C receptors between skin and lungs, despite both being endothelial tissues in origin.

5.4.16 Summary

The information generated by the several surveys conducted reveals several factors about the interaction of humans at Bribie Island undertaking MRWA, in an area know to have toxic *L. majuscula* present.

- High numbers (25%) of respondents reported skin symptoms, with most being mild symptoms.
- Severe dermatitis occurred at rate of 2.7% of those entering the undertaking MRWA.
- An association was found between level of water exposure and the reporting of skin and eye symptoms.

- Younger people were more likely to report skin, eye, and fever and headache symptoms and this difference persisted after adjusting for level of exposure, suggesting they may be more susceptible.
- Females reported greater skin symptoms after adjusting for exposure level and age.
- The association of skin and eye symptoms with level of exposure was maintained after adjusting for age and sex.

6. Fraser Island Study

6.1 Introduction - Fraser Island Study

Fraser Island is the world's largest sand island located 300km north of Brisbane in South East Queensland, Australia (Figure 6.1). It is 120km long and the majority of recreational beaches are on the eastern (ocean) side. In recent years there has been anecdotal evidence of blooms of *L. majuscula* and subsequent exposure of marine recreational water users (O'Neil, *et al.*, 2000). The aim of this study was to examine first aid records from Fraser Island to ascertain if any deleterious health consequences had a similar timing to outbreaks of *L. majuscula* growth.

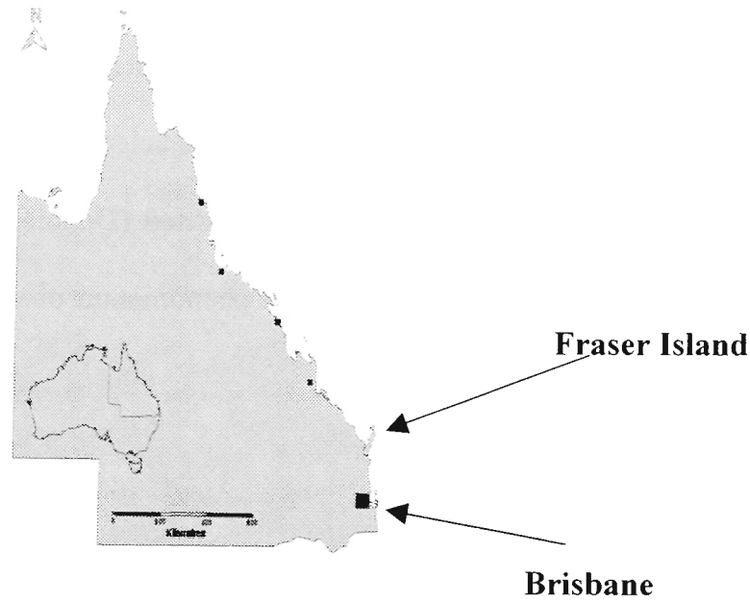


Figure 6.1 Location of Fraser Island on the Queensland Coast.

6.2 Methods - Fraser Island Study

6.2.1 Ethical Considerations

Ethical approval for this study was sought and gained from the Behavioural and Social Sciences Ethical Review Committee, University of Queensland (number B/169/NRCET/SOCPREVMED/99/PHD).

6.2.2 Data Collection

Access to first aid records from Fraser Island was obtained by the *Freedom of Information Act* (Government of Queensland, 1991). Information recorded by the Queensland National Parks Service rangers included date of birth, date of incident, postcode or country of origin of patient, location where treated, sex, occupation, notes on type or symptoms of injury, and medication or action was taken for the years including 1997-2001. These years were selected as they were around the summer of 1998 when anecdotal cases were reported (Dennison *et al.*, 1999), and as *Lyngbya* was assumed at this stage to be a phenomenon of warmer weather near the summer solstice.

6.2.3 Case Definitions

Cases of *Lyngbya*-like symptoms were identified subjectively based on the reporting of symptoms in the first aid report. Criteria used to classify symptoms as potentially due to *L. majuscula* in first aid reports of included pain, rash, redness, itching, burning, sore, running nose, sneezing, discharge, swelling, tenderness, slight dry cough, puffiness, inflammation, blister and irritation. These cases were included if they also affected specific tissue or organ of the body including groin, skin, upper respiratory tract and eyes. Some notes also mentioned commencement of symptoms

beginning after swimming in sea or exposure to onshore winds. Some cases actually mentioned symptoms were thought to be due to "fireweed" a colloquial term for *L. majuscula.*

6.2.4 Data Entry and Data Clearing

All data was originally entered into Microsoft Excel database Microsoft (1997b). Data was assessed for any replication of records. Data was then copied to Microsoft Access (Microsoft, 1997a) and SPSS (SPSS, 2002) for analysis.

6.3 Results - Fraser Island Study

There were 176 recorded presentations to first aid stations were present for the dates shown in Table 6.1. The majority (81.0%) of *Lyngbya*-like symptoms occurred in a seven-week period in January and February 1998. Examination of symptoms recorded by QNPS staff of attendees to first aid stations on Fraser Island over the four-year period examined found the highest number were physical injuries such as vehicle accidents, broken bones, strains, sprains and bruisings. The second highest report was the 21 persons reporting *Lyngbya*-like symptoms (11.9%). Other high numbers of people reported dingo bites, insect, scorpion and spider bites and burns. Fireweed was mentioned as a potential cause in five reports. Of the other reports, sixty (44.3%) cases were injuries of a physical nature. These included reports such as injuries sustained in vehicular accidents, broken limbs or sprains. Others excluded included bites, burns (from hot objects) or gastrointestinal problems (Table 6.1).

Records of the sex of persons reporting symptoms was predominately not undertaken with only 103 of 176 recording sex. Of the 21 recording *Lyngbya*-like symptoms 3 were recorded as male and 4 female. Due to the low amounts of data on the gender of individuals, no conclusion could be drawn.

Ages of those seeking first-aid treatments ranged from 1 to 71 years old (average 28.4 years). Of these 21 contained symptoms that could be related to exposure to *Lyngbya* with ages ranging from 20 to 48 (average 28.9 years). Seven (33.3%) were treated with ice packs. Other treatments included Loratidine (10mg/day), 1% Hydrocortizone cream (topically four times daily). No cases required patients to leave the island for further medical assistance.

Table 6.1 Reporting of symptoms at QNPS first-aid stations, Fraser Island.

incident	number of people
physical	60
***Lyngbya* -like symptoms**	21
dingo bites	18
other bites/stings	14
burns	11
stomach pains/vomiting	8
ear	5
infection	5
eye	3
blue-bottles	2
heart attacks	2
asthma	3
other	24
total reports	176

Table 6.2 176 first aid records were present for the following dates.

Timespan			No. Reports	No. Cases	%
November 1997	-	May 1998	105	19	18.1
November 1998	-	May 1999	37	1	2.7
November 1999	-	May 2000	22	1	4.5
November 2000	-	January 2001	4	0	0
unknown			8	1	12.5

6.3.1 Location on Island

Injuries with *Lyngbya*-like symptoms were from the Champagne Pools, Eurong and Dundubara on the ocean side of the island (7 missing location). Notably

Waddy Point, despite having high numbers of first aid patients recorded, had no *Lyngbya*-like symptoms recorded.

Table 6.3 Areas of Fraser Island recording symptoms of QNPS first aid records.

	Lyngbya -like symptoms	other symptoms	% *Lyngbya* - like	Map Number
Ngkala Rocks		1	-	1
Ocean Lake		1	-	2
Waddy Point		49	-	4
Champagne Pools	7	11	38.9	5
Indian Head		5	-	6
Dundubara	4	6	40	7
Lake McKenzie		3	-	9
Lake Wabby		1	-	10
Eurong	3	20	13	11
Dilli Village		10	-	12
Platypus Bay		1	-	
Poyungan Rocks		2	-	
Second Valley		1	-	
Semaphore Point		1	-	
missing location	7	43	16.3	
total	21	155		

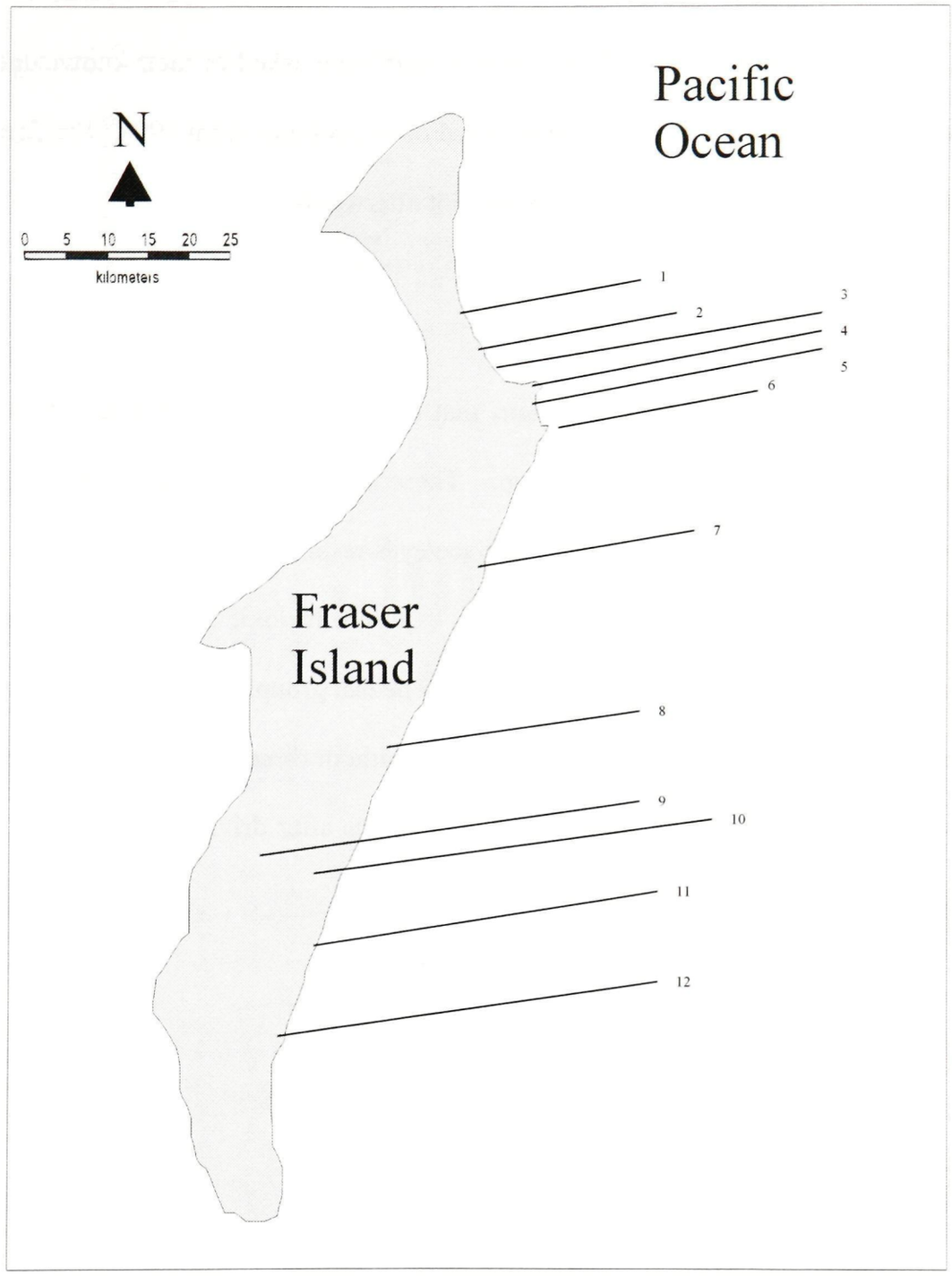


Figure 6.2 Fraser Island: 1. Ngkala Rocks; 2. Ocean Lake; 3. Orchid Beach; 4. Waddy Point; 5. Champagne Pools; 6. Indian Head; 7. Dundubura; 8. Happy Valley; 9. Lake McKenzie; 10. Lake Wabby; 11. Eurong; 12. Dilli Village.

6.3.2 Presence of *L. majuscula*

Queensland National Parks Service staff were asked of their knowledge of *L. majuscula* and when they had seen or heard of its presence from 1997. Staff reported that it had been present in early 1998 and not afterwards.

6.3.3 Symptoms

Individuals reporting symptoms that could be related to exposure to toxic *L. majuscula* fell into three distinct groups. These included those that reported injury to the groin, the skin on the torso, and face/eyes/respiratory tract. The former two groups were presumed to have had direct exposure to toxic *L. majuscula* (although only one noted symptoms after swimming). The last group were thought to have been exposed to aerosolised *L. majuscula,* perhaps without direct exposure to water. Two individuals mentioned the occurrence of symptoms after driving up the beach in sea spray.

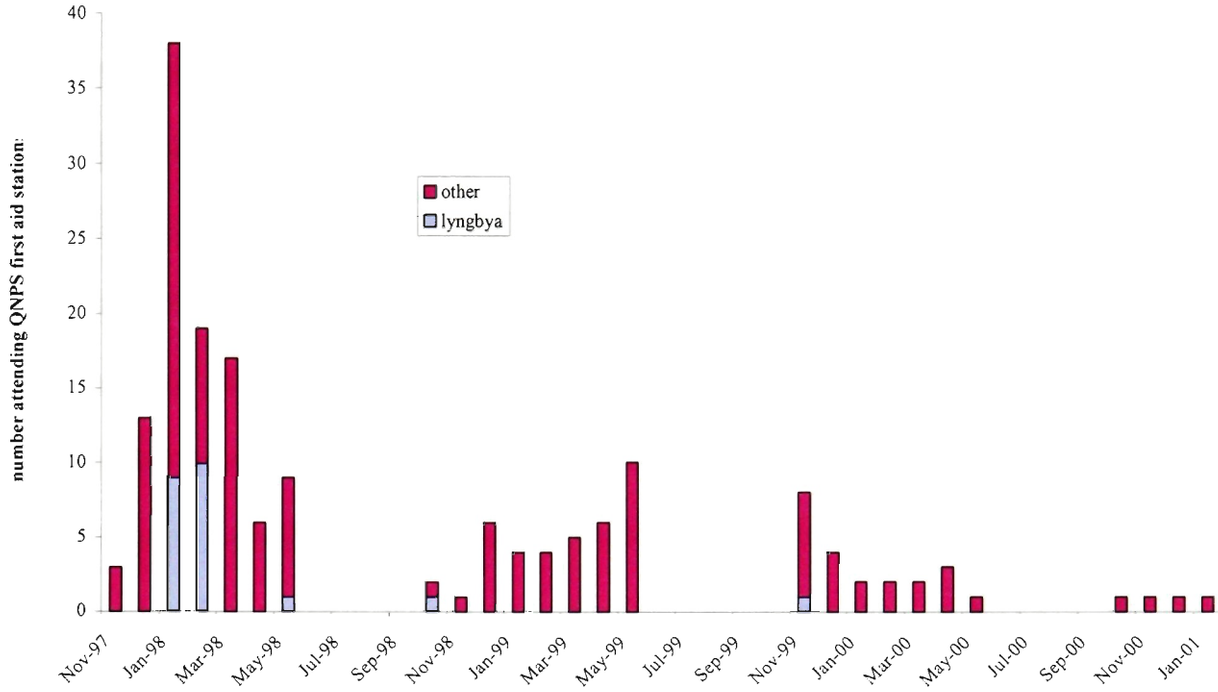


Figure 6.3 Reports of *Lyngbya*-like symptoms during the study period.

6.4 Discussion - Fraser Island Study

6.4.1 Symptoms

The types of symptoms displayed by individuals included in the *Lyngbya*-like symptoms group were similar to those reported by other authors. In the first aid record notes 9 of the twenty-one cases had included injuries that were in the inguinal region. This has been noted as classic symptoms of *Lyngbya*-related exposure whilst swimming (Grauer *et al.*, 1961; Hashimoto *et al.*, 1976). Another 8 cases reported symptoms common after exposure to aerosolised *L. majuscula* as reported by other authors (Hashimoto *et al.*, 1976; Anderson *et al.*, 1988). A further 4 cases reported symptoms of skin itchiness, rash and irritation, similar to those to be expected after MRWA in waters containing toxic *L. majuscula* (Izumi *et al.*, 1987).

6.4.2 Temporal Aspects

The vast majority of reports of *L. majuscula*–like symptoms were in a seven-week period of January and February 1998. A third of reported cases occurred on a single day in the middle of this period. This type of short time span of reporting is typical of *L. majuscula* toxic incidents. Of the six outbreaks of dermatitis related to *L. majuscula* reported in the literature, the majority occurred over a small time period during the year. Some reports are limited in information, only give a range of time periods over a two month period (Grauer *et al.*, 1961), while others are more specific, nominating a single day (Hashimoto *et al.*, 1976). Others give a range but nominate a day or two where the majority of cases occur. Reasons for this are two fold. The majority of dermatitic events occurred in a one to two month period after the summer solstice (northern or southern hemisphere, respectively). At this time the seawater has received maximal length of sunlight and began to warm. The *L. majuscula* has

received higher amounts of light to grow. This growth may, in turn, trigger toxin production. With the increasing air and water temperatures, increased numbers of individuals are undertaking MRWA. The summer period in subtropical/tropical regions is storm season where may lead to strong onshore winds and water condition where *L. majuscula* is broken up and enters the water column. A combination of these factors leads to an outbreak of dermatitis caused by *L. majuscula*, with the majority of epidemics causing serious injuries effecting larger numbers of people only occurring at a single time at a single location (Table 6.4). An alternative hypothesis is that *L. majuscula* growing in Hervey Bay is taken around Sandy Cape by tidal flow and is then picked up by the East Australian current which flows south along the eastern coast of the island. The *L. majuscula* is then subsequently deposited on the ocean beaches of Fraser Island. The rarity of events conspiring towards an outbreak of dermatitis serious enough to warrant visits to health professionals is exemplified by only six events being reported in the literature in 45 years.

Table 6.4 Reported events of *L. majuscula* toxicity in humans.

date	Location	no. cases	author	time period	maximum period	days from solstice
1958	Oahu, Hawaii	125	Grauer and Arnold, 1961	July- August		+8-70
1959	Oahu, Hawaii		Grauer and Arnold, 1961	late June- July		+8-39
1960	Oahu, Hawaii		Grauer and Arnold, 1961	late June- July		+8-39
1968	Gushikawa, Okinawa	242	Hashimoto *et al.*, 1976	21 July	late afternoon	+29
1976	Oahu, Hawaii		Mynderse *et al.*, 1977	September		
1980	Oahu, Hawaii	86	Serdula *et al.*, 1982	13-26 August	18 August	+57
1983	Maui, Hawaii	31	Anderson *et al.*, 1988	29-31 July	18:00-22:00 hours	+38
1986	Oahu, Hawaii		Izumi and Moore, 1987	summer		
1998	Fraser Island, Australia	21	this study	January-Februrary	7 February	+47

6.4.3 Spatial Aspects

Sites where *Lyngbya*-like symptoms were reported were all on the ocean (eastern) side of the island. These included Champagne Pools, Dundubara and Eurong. Several factors would lead to increased incidence of *L. majuscula*-related symptoms on the eastern coast including the presence of *L. majuscula*, presence of individuals undertaking MRWA and meteorological conditions leading to the interaction of these two.

The majority of people are on the eastern (ocean) beach on Fraser Island. Much of the western coastline of Fraser Island is not used for recreational purposes. Several factors contribute towards this. The main avenue of transport from the south to the north of the island is the Seventy-Five Mile Beach (also used as a landing strip for aircraft), on the eastern side of the island. The main settlements on the island are concentrated of this side, including ranger and first aid stations. The coastline on the western side of the island is often swampy and/or populated by mangroves, and has considerable populations of mosquitoes and sand flies. These factors make it unpopular with both the tourist and recreational fishers.

The Champagne Pools, bubbling seawater rock pools, are recommended as a swimming location (Sunmap, 1998). It is popular among adults with children for this reason probably due to their calmness, compared to the surf on the ocean beaches, and higher temperature. This is also the location where staff of the Queensland National Parks Service reported *L. majuscula*, and where signs were erected to notify the presence of "harmful algae". Others have reported anecdotal evidence on *L. majuscula*-like symptoms after women and children bathing in 'beach pools', formed by a hump of sand on the beach and pools forming behind them, which were warm during the day (Buchanan Heritage Services, 2003).

The prevailing winds in the area are south easterly making the eastern side of the island the windward side. Several authors have reported the presence of *L. majuscula* on the windward side of coastlines in diverse geographical locations. Oahu, Hawaii has been noted by several authors in 1958, 1959 and 1960 (Banner, 1959; Grauer *et al.*, 1961) and 1980 (Sims *et al.*, 1981). All six beaches where dermatitis has occurred were on the windward side (Laie Bay, Kaawa, Kaneohe Bay, Kailua Bay, Lanikai and Waimanalo Beach)(Grauer *et al.*, 1961). Toxic incidents purported to have their origin in *L. majuscula* occurred during onshore winds on the island of Maui, Hawaii, soon after tropical storms (Anderson *et al.*, 1988). Strong winds sweeping over the shore were present during an outbreak of dermatitis linked to *L. majuscula* in Gushikawa Beach, Okinawa in1968 (Izumi *et al.*, 1987). A small outbreak of 'seaweed' dermatitis on Oahu in 1986 was on the windward coast (Izumi *et al.*, 1987). Toxic incidents related to *L. majuscula* occurred on the windward side of Fraser Island (Champagne Pools, Dundubura and Eurong).

'Lyngbya' has been reported in Hervey Bay by numerous fishers during the last century (Buchanan Heritage Services, 2003). Although the western side of Fraser Island is adjacent to this bay, no reports of *Lyngbya*-like symptoms came from this area by recreational water users. This poses this question that perhaps the increased reporting of *Lyngbya*-like symptoms is due to increased numbers of recreational water users rather than an increase in toxicity, or bloom size and longevity of *L. majuscula.* This increase coincides with increases in Queensland of the population, MRWA, tourism and use of 4-wheel drive vehicles and research into this organism.

6.4.4 Summary

A number of individuals attended Queensland National Parks Service first aid station on Fraser Island with symptoms synonymous with exposure to toxic *L. majuscula*. The majority of these cases occurred in a seven-week period in the summer of 1998. This was also the only period that ranger of the Queensland National Parks Service identified the presence of *L. majuscula* on Fraser Island.

7. General Discussion of Findings in this Thesis

In this thesis it was attempted to give an assessment of human health risk of the cyanobacterium *L. majuscula*. This has been pursued by using health risk assessment methodologies (World Heath Organization, 2000). A hazard was initially examined by the identification of cyanobacterium species and characterisation and quantification of toxin in this species. Initial anecdotal evidence of the presence of a "weed" that gave skin and eye irritation was noted in South East Queensland by Dennison *et al.* (1999). The species was taxonomically identified and literature revealed that it had been previously been found to contain irritants LA and DAT. Samples of *L. majuscula* were collected from Moreton Bay, Queensland for this thesis and were found, using LCMS, to contain LA and DAT.

A series of experiments were designed to examine the toxicology of *L. majuscula* in mammals, particularly dermal irritancy to assess the toxicity of *L. majuscula* found in Queensland waters (Chapter 3). Toxins LA and DAT were found to produce severe dermal irritancy to mouse skin at the μg amounts. The toxins LA and DAT were shown to be toxic in a dose dependant manner. Increasing amounts of both LA and DAT produced increasing irritancy in the mouse ear swelling test model of dermal irritancy. LA and DAT were found to produce similar irritancy at similar concentrations, and no synergistic effect was found between the toxins. All the toxicity of the Queensland *L. majuscula* was not explained by the presence of LA and DAT in samples, suggesting other toxins or modulating factors may also be present.

Examination of many field samples collected from a range of areas in South East Queensland show a range of concentrations of both DAT and LA (Chapter 4). The highest found was 132.9 mg/kg of DAT (freeze dried weight). The amount of toxin present in these samples was high enough to lead to skin irritation in humans, if

exposure occurred. Toxins quantities were found to vary both spatially and temporally in South East Queensland. Toxin concentrations in samples of *L. majuscula* within a single location varied by as much as ten-fold. This variation in toxicity was thought to be linked to environmental factors such as degree of growth of bloom and temperature. The influence of genetic factors has not been ruled out. This variation in toxin concentration in *L. majuscula* makes it impossible to estimate health risk with any certainty at any particular moment without monitoring every bloom individually. With more research, patterns of toxicity may emerge allowing estimations of risk to be made with greater accuracy.

In an attempt to better characterise the risk of *L. majuscula* to human health an assessment of exposure was made. It was not ethically or technologically possible to make an estimate of human exposure to LA and DAT by exposing individuals to toxic cyanobacterium while undertaking MRWA. In an attempt to investigate the potential exposure to LA and DAT a large population of marine recreational water users, in an area where toxic blooms of *L. majuscula* were know to occur, were questioned by postal survey. Questions concerned an individual's exposure and symptoms after MRWA. Higher water exposure was associated with increased likelihood of reporting of skin and eye symptoms. Information derived from this survey revealed only 2.7% of people experience severe skin symptoms over a seven month period. This data of low incidence symptoms in areas known to have toxic blooms of *L. majuscula* was supported with records from Fraser Island first aid stations. *L. majuscula* has been found periodically in the locality of Fraser Island (Buchanan Heritage Services, 2003). Up to 250 000 people visit the island every year. Despite these large numbers of individuals visiting the island, with many presumably undertaking MRWA, only 21 people were found to have reported *Lyngbya*-like symptoms in more than 3 summers.

These two studies data have revealed that the risk of significant acute symptoms is low. From this data it can be concluded that despite toxins found in *L. majuscula* being highly irritant, the low human exposure has made the risk of this organism compromising human health low. Furthermore it highlights that health risk assessment based only on animal data coupled with statistical extrapolation models may lead to false conclusions. Epidemiology strengthens laboratory data as it involves human populations in real-world conditions (World Health Organisation, 2000). The combination of animal toxicology data with epidemiological research allows a better model of health risk assessment to be put forward. This approach has been missing in many health risk assessment studies, making their validity questionable (Hertz-Picciotto, 1995).

The probable explanation of why such a toxic organism affects human health to only a small extent is the minimal degree of exposure to toxins. This study differs from all other studies on *L. majuscula* in that it is a prospective study examining a population living in an area that has toxic blooms of the marine cyanobacterium *L. majuscula* that has not experienced an "epidemic" of *L. majuscula*-associated symptoms. "Epidemic" is used here in the context of large numbers of cases occurring in a short space of time, with in a specified area. Only small numbers of case reports of exposure to cyanobacteria have been published (<20) and only a few of these relate to *L. majuscula* (Table 7.1). Most of these lack information and many are confined to a report that irritation occurred in a small number of bathers. No such "epidemic" on the scale of published reports appears to have occurred in the period covered by the survey despite toxic *L. majuscula* being present.

Table 7.1 Documented occurrences skin, eye and respiratory irritation by *L. majuscula.*

Date	Location	Country	No. cases	Author
1958	Laie	Hawaii	123	Grauer *et al.*, (1961)
1958	Kaaawa	Hawaii		Grauer *et al.*, (1961)
1958	Kailua Bay	Hawaii		Grauer *et al.*, (1961)
1958	Waimanalo Beach	Hawaii		Grauer *et al.*, (1961)
1959	Laie	Hawaii		Grauer *et al.*, (1961)
1960	Laie	Hawaii		Grauer *et al.*, (1961)
1968	Okinawa	Japan	242	Hashimoto *et al.*, (1976)
1973	Okinawa	Japan		Hashimoto *et al.*, (1976)
1976	Laie Bay, Oahu	Hawaii		Mynderse *et al.*, (1977)
1980	Kailua, Oahu	Hawaii	86	Serdula *et al.*, (1982)
1980	Kalama, Oahu	Hawaii		Serdula *et al.*, (1982)
1980	Pilapu, Oahu	Hawaii		Serdula *et al.*, (1982)
1983	Maui	Hawaii	31	Anderson *et al.*, (1988
1986	Oahu	Hawaii		Izumi *et al.*, (1987)
1996-7	Moreton Bay	Australia		Dennison *et al.*, (1999)
1997-8	Moreton Bay	Australia		Dennison *et al.*, (1999)

Large differences in severity of health outcomes after exposure to *L. majuscula* have been noted, from tingles and mild itching to skin blistering over whole limbs and torso. Only exposures that have led to severe health outcomes would have been reported in the literature. No occupational injury from exposure to *L. majuscula* was reported before 1999 (Dennison *et al.*, 1999). This area may provide some answers but information derived from this must be assessed closely as political pressure on and by the fishing industry may lead to large degrees of bias.

Epidemic outbreaks of *L. majuscula* occur when a certain series of events take place involving humans and *L. majuscula,* that eventually leads to the exposure of numbers of humans to toxins contained in this species. A schematic model of potential events leading up to outbreaks seen in Queensland and other locations in the world have been included in Figure 7.1. If this model is correct, this may reflect the rarity of reports of adverse health effects due to *L. majuscula* as many variables must be present to facilitate human exposure to toxins produced by this cyanobacterium.

Possible explanations of why outbreaks of severe dermatitis do not always occur in areas that have the potential for *L. majuscula* toxins affecting people who undertake MRWA are given below. Climatic conditions in areas that are susceptible to *L. majuscula* outbreaks must be appropriate for a bloom of *L. majuscula* to occur. The model below describes a certain sequence of climatic events that needs occur prior to large-scale injuries to humans undertaking MRWA. Any break in this sequence of events would not produce large numbers of irritations amongst individuals undertaking MRWA. Many factors considered essential for the blooming of benthic cyanobacteria such as nutrient loading of phosphorus, iron and other nutrients can be linked to rainfall and runoff from catchment areas surrounding *L. majuscula* habitats. Increasing global temperature may also lead to a shift in the ecological make up of biological communities, which may include the increasing harmful algal blooms (van Dolah, 2000). This may in part be due to increased rainfall in areas marginal to cyanobacterial blooms, allowing species to inhabit niches previously unavailable to them. Much evidence has now accumulated, in a diverse range of environments, to show that ecological change is occurring due to recent climate change, from species to the community level (Walther *et al.*, 2002).

Cyanobacteria have been found to bloom under weather conditions that are both calm and hot. *L. majuscula* blooms have been found to be initiated after prolonged high surface light (44.7 mol quanta $m^{-2}s^{-1}$), following a rainfall event (Watkinson, 2000). Bloom formation of *Trichodesmium erythaemum* has been found to increase in periods of bright sunshine after rain (Sato *et al.*, 1966). Calm weather conditions also result in high light penetration (mean K_d-0.77 m^{-1}) and constant near full strength salinity (34.8 ppm), are prerequisite for blooms in North Deception Bay (South East Queensland Regional Water Quality Management Strategy, 2001). Water

temperature has been found to be a major component in growth of cyanobacteria (Robarts *et al.*, 1987; Sivonen, 1996). Stillness in the water column has been found to be advantageous in growth of microalgae including cyanobacteria, while growth of algal turf comprising small filamentous algae and cyanobacteria in the marine environment has been noted to be compromised by physical disturbances such as wave action (Cheroske *et al.*, 2000). In fact, physical disturbance has been found to be a more important influence on cyanobacterial abundance and distribution (including marine Pacific *Lyngbya* spp.) than either nutrient availability or interactions with macroalgae (Thacker *et al.*, 2001). Benthic mats of *L. majuscula* must have clear water, in which sunlight penetrates to the bottom for growth and this is in turn dependent on the clear weather conditions.

Combined with positive growth of *L. majuscula*, humans are more likely to enter the water during warmer periods. Similar correlations between temperature and bloom and temperature and number of people entering the water recreationally in temperate climates involving freshwater cyanobacteria have been made (Chorus *et al.*, 2000).

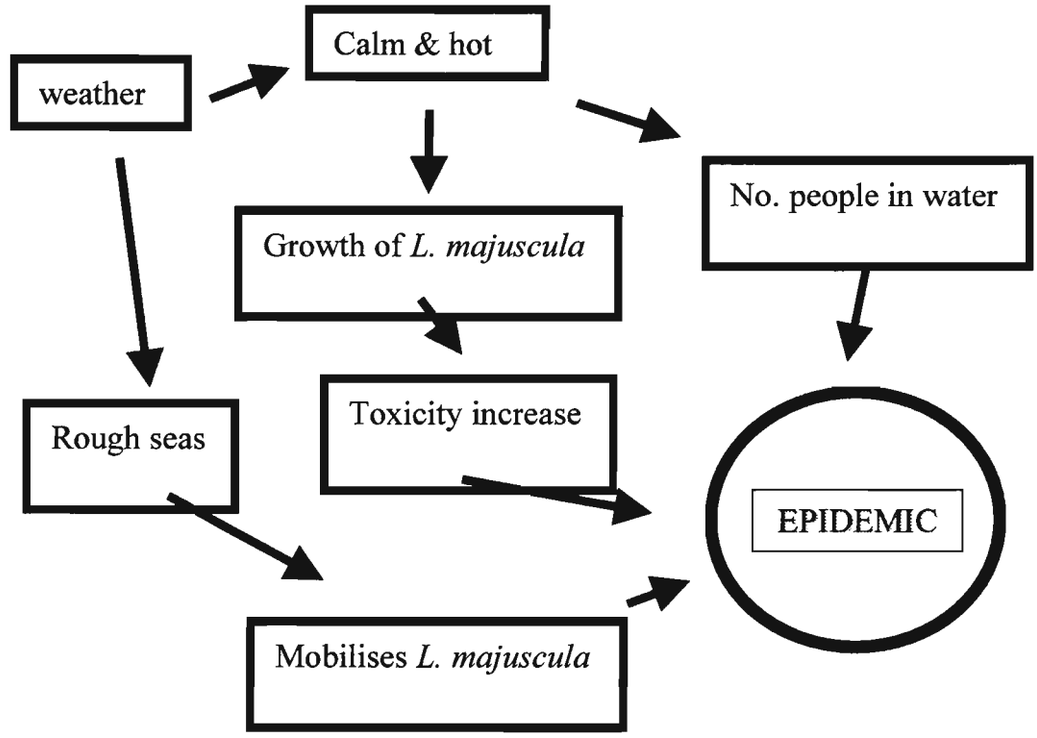


Figure 7.1 Schematic model of linkages of important factors in the "epidemic" of negative health outcomes in relation to exposure to *L. majuscula*. Such linkages may also be important for other benthic and non-benthic cyanobacteria.

Combined with positive growth of *L. majuscula*, humans are also more likely to enter the water during warmer periods. Correlations have been made between temperature and bloom and temperature and number of people entering the water recreationally in temperate climates involving freshwater cyanobacteria (Chorus *et al.*, 2000). Larger numbers of people are also participating in MRWA in the Bribie Island area, especially in the last 20 years. A large increase in the population of areas surrounding Moreton Bay and surrounding areas has occurred in recent years with increases approximately 1.5-2% per annum (Brisbane City Council, 2003). Access to remote locations has increased with the recent increased availability and popularity of four-wheel drive vehicles allowing to access areas such as Fraser Island. Recreational use of boats, as well as personal watercraft, has also increased.

L. majuscula must be toxic as well as blooming for negative health outcomes. Toxins must also be in sufficient concentration to elicit an effect. For an "epidemic", high concentrations of toxins are required. By definition, acute (primary) irritant dermatitis, in contrast to allergic contact dermatitis, is elicited at first exposure (not in multiple exposure) in most of the population (Wilhelm *et al.*, 1990). It is the first exposure that is important and that must be high enough to cause injury or people just avoid the area without having major irritation, just minor itching and they stay out of the water. This will be different in individuals as irritant threshold and dose responses vary considerably among individuals when tested with mild irritants (Frosch *et al.*, 1980; Frosch *et al.*, 1977; Frosch *et al.*, 1982; Wilhelm *et al.*, 1989).

Data in this thesis shows that production of the irritant toxins LA and DAT is both spatial and temporal in nature. Furthermore, production of high amounts of toxin may occur at the bloom peak. This has also been found in some freshwater cyanobacteria (Sivonen, 1996). These spatial and temporal differences in toxin production will further decrease the likelihood of an epidemic occurring.

Being benthic, *L. majuscula* may not be a large health problem *in situ* as human exposure would be minimal. Loosely attached to coral, seagrass or macroalgae, mats may persist for months (South East Queensland Regional Water Quality Management Strategy, 2001). During periods of high sunlight, it has been noticed to float up off the bottom, probably due to oxygen production at high rates of photosynthesis, an alteration in buoyancy somewhat similar to the marine cyanobacterium *Trichodesmium* (Romans *et al.*, 1994). Once it enters the water column it is at the mercy of currents and tides, which may or may not move *L. majuscula* to areas where MRWA is occurring. Other toxic events due to benthic

species of cyanobacteria have been noted to follow similar series of events leading to mobilisation (Edwards *et al.*, 1992; Mez *et al.*, 1996).

Rough weather after a period of calm weather will also have the same effect, with *L. majuscula* entering the water column and being mobilised, increasing the chance of it moving toward areas used for MRWA. Epidemics of seaweed dermatitis have usually only been reported on the windward side of Oahu, Hawaii, during summer months (Grauer *et al.*, 1961). At an enclosed bay on this side near Kailua Beach, near where reports were first made in 1958, no *L. majuscula* or outbreaks of dermatitis were reported, emphasising that exposure to *L. majuscula* is dependant on the meteorological conditions and the surrounding geographical environment. During the outbreak of dermatitis at Gushikawa Beach in 1968, a strong wind had been blowing onshore, causing much ocean spray leading to eye irritation (Hashimoto, 1979). Anderson *et al.*, (1988) documented that the reports of adverse health outcomes associated with *L. majuscula* exposure coincided with periods of high surf from Tropical Storm "Gil" and high tides. Such weather conditions of warm periods followed by storms are synonymous with subtropical/tropical environments. Ocean upwelling, caused by *El Niño* or hurricanes has been proposed to increase risk of intoxication by the benthic toxic marine dinoflagellate *Prorocentrum lima* (Heredia-Tapia *et al.*, 2002). The incidence of human respiratory irritation, associated with high concentrations of *Gymnodinium* sp., correlated with the increase in wind, braking waves and foam and the presence of small drops of red water in the air (Woodcock, 1948). Aerosolised fragments sent over the beach of the marine cyanobacterium *Trichodesmium* sp. in Brazil have been postulated to be the source of rash, respiratory irritation and fever (Sato *et al.*, 1966). These symptoms occurred on windy days but disappeared with the onset of rain, presumably as the rain cleared the air of aerosols.

Izumi *et al.* (1987) presumed that the strong currents and winds during these periods dislodged *L. majuscula* from the ocean floor and pulverised it into small fragments in the surf. These fragments then blew to shore or lodged in the trunks of swimmers. This differs in the scenarios usually played out recently in Northern Deception Bay. Large wracks of whole *L. majuscula* strands accrued on beaches there with 400 tonnes cleared from beaches in the summer of 2000-2001. The Bribie Island Survey began during this period. Different aetiologies of the disease process maybe due to the differential weather condition or topography. North Deception Bay is a shallow estuarine system on the edge of a continent while Hawaiian geography is volcanic nature and in the middle of the Pacific Ocean.

Measurements taken of seawater surrounding a large bloom of *L. majuscula* revealed no toxins present on 22 January 2001, the beginning of the Bribie Island Survey period (Chapter 4). This suggests that the toxin release may require either direct contact with *L. majuscula* or breaking of the cell wall to release toxins. Rough seas may play a role in this, and exposure of *L. majuscula* to strong sunlight after it has become detached may reduce toxicity. Hashimoto (1979) reports that the toxicity of *L. majuscula* was decreased by half after exposure to ultraviolet radiation for three hours.

Rough weather has also been associated with aerosolisation of toxins from *L. majuscula* on at least two separate occasions in Asia and the Pacific regions (Hashimoto, 1979; Anderson *et al.*, 1988). The accumulation of wracks of *L. majuscula* on the beach at Fraser Island was also dependent on weather conditions and tides etc. Once it had been deposited on the beach four-wheel drive vehicles driving over it may have lead to it becoming aerosolised and causing eye and respiratory irritation (QPWS, 2001).

The information generated by a literature review, experimental work and the several surveys conducted for this thesis reveals several factors about the interaction of humans with *L. majuscula.* Many of these conclusions are reflected in the knowledge gained by other researchers in other locations in the world.

- Toxins in *L. majuscula* vary spatially and temporally.
- Other, as yet unknown factors could be affecting the toxicity of *L. majuscula.*
- Outbreaks of irritation associated with *L. majuscula* blooms are episodic.
- A potential reason for these outbreaks being episodic is meteorological.
- High numbers of respondents (26%), particularly young people and females, complained of skin complaints after engaging in MRWA in Moreton Bay.
- Severe outbreaks of irritation are rare (2.7%), but were more common among young people and females.

8. Bibliography

Abal E.G., Dennison W.C. and Greenfield P.F. (2001) Managing the Brisbane River and Moreton Bay: an integrated research/management program to reduce impacts on an Australian estuary. *Water Sci. Technol.*, 43: 57-70

ABS (1998) National Health Survey. Asthma and other respiratory conditions, Australia, 1995. Australian Bureau of Statistics, Canberra.

ABS (2001) 1996 Census of Population and Housing Bribie Island (Statistical Local Area) - Queensland Australian Bureau of Statistics. [www.abs.gov.au].

Agner T. and Serup J. (1993) Time course of occlusive effects on skin evaluated by measurement of transepidermal water loss (TEWL). *Contact Dermatitis*, 28: 6-9

Agner T., Damm P. and Skouby S. (1991) Menstrual cycle and skin reactivity. *J. Am. Acad. Dermatol.*, 24: 566-570

Aguilera D., L. F. (1973) [Clinical resemblance of Oppenheim's contact dermatitis and dermatitis produced by contact with marine algae impregnated with urticating cells of Physalia arethusia]. *Ann. Dermatol. Syphiligr. (Paris).* 100: 25-7

Aguirre A., Limpus C., Spraker T. and Balazs G. (1999) Survey of fibropapillomatosis and other potential diseases in marine turtles from Moreton Bay, Queensland, Australia. The 19th Annual Symposium on Sea Turtle Biology and Conservation. pp. 36

Aimi N., Odaka H., Sakai S., Fujiki H., Suganuma M., Moore R.E. and Patterson G.M. (1990) Lyngbyatoxins B and C, two new irritants from *Lyngbya majuscula*. *J. Nat. Prod.*, 53: 1593-6

Ainslie R.D., Moore R.E. and Patterson G.M.L. (1986) (S)-(levo)-3,4-Dihydroxybutanoic acid gamma-lactone from Puerto Rican *Lyngbya majuscula*. *Phytochemistry*, 25: 2654-2655

Albert S. (2002) *The Effects of Soil Extracts on the Physiology of Lyngbya majuscula (Cyanophyta)* Honours Thesis, University of Queensland.

Anagnostidis K. and Komarek J. (1988) Modern approach to the classification system of cyanophytes 3 - oscillatoriales. *Arch. Hydrobiol. (Suppl.)*, 80: 327-472

Andersen P. (1996) *Design and Implementation of Some Harmful Algal Monitoring Systems*. IOC Technical Series No.44. UNESCO

Anderson B., Sims J., Liang A. and Minette H. (1988) Outbreak of eye and respiratory irritation in Lahaina, Maui, possibly associated with *Microcoleus lyngbyaceus*. *J. Environ. Health*, 50: 205-209

Anon. (88/89) Swimmers' itch, a surfacing problem? An outbreak at a Suffolk watersport park. *Comm. Dis. Intell.*, 88/89: 3-6

Australia and New Zealand Environmental and Conservation Council (1992) Australian Water Quality Guidelines for Fresh and Marine Waters. Australian and New Zealand Environment and Conservation Council, Canberra.

Australia and New Zealand Environmental and Conservation Council (2000) Australian guidelines for water quality monitoring and reporting. Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra.

Appleton C.C. and Lethbridge R.C. (1979) Schistosome dermatitis in the Swan Estuary, Western Australia. *Med. J. Aust.*, 1: 141-5

Appleton D., Sewell M., Berridge M. & Copp B. (2002) A new biologically active malyngamide from a New Zealand collection of the sea hare *Bursatella leachii*. *J. Nat. Prod.*, 65: 630-631

Arcoleo J.P. and Weinstein I.B. (1985) Activation of protein kinase C by tumor promoting phorbol esters, teleocidin and aplysiatoxin in the absence of added calcium. *Carcinogenesis*, 6: 213-218

Armstrong J.E., Janda K.E., Alvarado B. and Wright A.E. (1991) Cytotoxin production by a marine *Lyngbya* strain (cyanobacterium) in a large-scale laboratory bioreactor. *J. Appl. Phycol.*, 3: 277-282

Arnarson E., Gudmundsdóttir A. and Boyle G. (1998) Six-month prevalence of phobic symptoms in Iceland: An epidemiological postal survey. *J. Clin. Psychol.*, 54: 257-265

Arnold H. (1959) Seaweed dermatitis apparently caused by a marine alga. Clinical observations. Proceedings of the 34th Annual meeting of the Hawaiian Academy of Sciences pp. 20

Asch D., Jedrwiewski M. and Christakis N. (1997) Response rates to mail surveys published in medical journals. *J. Clin. Epidemiol.*, 50: 1129-1136

Asherson G. and Ptak W. (1968) Contact and delayed hypersensitivity in the mouse. I. Active sensitization and passive transfer. *Immunology*, 15: 405-416

Auerbach P. (1987) Natural microbiological hazards of the aquatic environment. *Clin. Dermatol.*, 5: 52-61

Auerbach P.S. and Hays J.T. (1987) *Erythema nodosum* following a jellyfish sting. *J. Emerg. Med.*, 5: 487-91

Australian Electoral Commission (2001) Elector extract by age range for the Federal Division on Longman as of 31 May 2001, Australian Electoral Commission, Canberra.

Baden D., Mende T., Poli M. and Block R. (1984) Toxins's from Florida's red tide dinoflagellates, *Ptychodiscus brevis*. In: *Seafood Toxins* (Ed. Regelis E.). American Chemical Society, Washington, D.C., pp. 359-367

Baker P.D. and Humpage A.R. (1994) Toxicity associated with commonly occurring cyanobacteria in surface waters of the Murray-Darling Basin, Australia. *Aust. J. Mar. Fresh. Res.*, 45: 773-786

Banner A.H. (1959) A dermatitis-producing algae in Hawaii. *Hawaii Med. J.*, 19: 35-36

Banner A.H., Scheuer P., Sasaki S., Helfrich P. and Alender C. (1960) Oberservations on ciguatera-type toxin in fish. *Ann. NY Acad. Sci.*, 90: 770-787

Bartram J., Carmichael W.W., Chorus I., Jones G. and Skulberg O.V. (1999) Introduction. In: *Toxic Cyanobacteria in Water* (eds. Bartram J and Chorus I). E and FN Spon, London, pp. 1-14

Baurle G., Hornstein O. and Diepgen T. (1985) Occupational hand eczema and atopy. *Dermatosen*, 33: 161-165

Bell S. and Codd G. (1994) Cyanobacterial toxins and human health. *Rev. Med. Microbiol.*, 5: 256-264

Beltran E. and Neilan B. (2000) Geographical segregation of the neurotoxin-producing cyanobacterium *Anabaena circinalis*. Appl. Environ. Microbiol. 66: 4468-4474

Berardesca E. and Distante F. (1994) The modulation of skin irritation. *Contact Dermatitis*, 31: 281-287

Berman F.W., Gerwick W.H. and Murray T.F. (1999) Antillatoxin and kalkitoxin, ichthyotoxins from the tropical cyanobacterium *Lyngbya majuscula*, induce distinct temporal patterns of NMDA receptor-mediated neurotoxicity. *Toxicon*, 37: 1645-1648

Best J., Pflugmacher S., Wiegand C., Eddy F. and Codd G.A. (2000) The effect of lipopolysaccharide and microcystin-LR on glutathione S-transferase activities in fish. 9th International Conference on Harmful Algal Blooms Hobart, Australia pp. 7

Best J., Pflugmacher S., Wiegand C., Eddy F., Metcalf J. and Codd G.A. (2002) Effects of enteric bacterial and cyanobacterial lipopolysaccharides, and of microcystin-LR, on glutathione *S*-transferase activities in zebra fish (*Danio rerio*). *Aquatic Tox.*, 60: 223-231

Best W. and Sablan R. (1964) Cymothoidism (sea louse dermatitis). *Arch. Dermatol.*, 90: 177

Beutler J.A., Alvarado A.B., Schaufelberger D.E., Andrews P. and McCloud T.G. (1990) Dereplication of phorbol bioactives: *Lyngbya majuscula* and *Croton cuneatus*. *J. Nat. Prod.*, 53: 867-881

Bickers D., Dutta-Choudhury T. and Mukhtar H. (1982) Epidermis: a site of drug metabolism in neonatal rat skin. Studies on cytochrome P-450 content and mixed-function oxidase and epoxide hydroxylase activity. *Mol. Pharmacol.*, 21: 239-247

Bjornberg A. (1975) Skin reactions to primary irritants in men and women. *Acta Derm. Venereol.*, 55: 191-194

Bloch C., Blackburn S., Jones G., Orr P. and Grewe P. (1997) Plasmid content and distribution in the toxic cyanobacterial genus Microcystis Kutzing ex Lemmermann (Cyanobacteria: Chroococcales). *Phycologia*, 36: 6-11

Bolumar F., Barros H., Florey C., Olsen J., Osler M., Skjaerven R., Diaz M.J.T. and Zielhuis G. (2002) Good Epidemiological Practice International Epidemiologist Association/European Epidemiological Group. URL)|. Pub

Bond J. and Barry B. (1988) Hairless mouse skin is limited as a model for assessing the effects of penetration enhancers in human skin. *J. Invest. Dermatol.*, 90: 810-813

Bonnard I., Rolland M., Francisco C. and Banaigs B. (1997) Total structure and biological properties of laxaphycins A and B, cyclic lipopeptides from the marine cyanobacterium *Lyngbya majuscula*. *Lett. Peptide Sci.*, 4: 289-292

Brand L.E., Sunda W.G. and Guillard R.R.L. (1986) Reduction of marine phytoplankton reproduction rates by copper and cadmium. *J. Exp. Mar. Biol. Ecol.*, 96: 225-250

Brand-Gardner S., Lanyon J. and Limpus C. (1999) Diet selection by immature green turtles, *Chelonia mydas*, in subtropical Moreton Bay, south-east Queensland. *Aust. J. Zool.*, 47: 181-191

Brisbane City Council (2003). Estimated Resident Population. [www.brisbane.qld.gov.au/business/economic_trends/key_indicators/population.shtml]: Brisbane City Council.

Bruce N. (1987) Australian Renocila Miers, 1880 (Isopoda: Cymothoidae), crustacean parasites of marine fishes. *Records of the Australian Museum*, 39: 169-182

Buchanan Heritage Services (2003) *Lyngbya* - History of Occurence. For Moreton Bay Waterways and Catchement Partnership, Brisbane, Australia.

Burja A., Abou-Mansour E., Banaigs B., Payri C., Burgess J. and Wright P. (2002) Culture of the marine cyanobacterium, *Lyngbya majuscula* (Oscillatoriaceae), for bioprocess intensified production of cyclic and linear lipopeptides. *J. Microbiol. Methods*, 48: 207-219

Burke W.A. (2002a) Cnidarians and human skin. *Dermatol Ther*, 15: 18-25

Burke W.A. (2002b) Skin problems from marine arthropods. *Dermatol Ther*, 15: 43-46

Burnett J., Calton G., Burnett H. and Mandojana R. (1987) Local and systemic reactions from jellyfish stings. *Clin. Dermatol.*, 5: 14-28

Burns C. (1987) Insights into zooplankton-cyanobacteria interactions derived from enclosure studies. *N.Z. J. Marine Freshwater Res.*, 21: 477-482

Bury N., Eddy F. and Codd G. (1995) The effects of the cyanobacterium *Microcystis aeruginosa*, the cyanobacterial hepatotoxin microcystin-LR, and ammonia on growth rate and ionic regulation of brown trout. *J. Fish Biol.*, 46: 1042-1054

Capone D.G. (1997) Microbial Nitrogen Cycling. In: *Manual of Environmental Microbiology* (Ed. Hurst C). ASM Press, Washington,

Cardellina J., Dalietos D., Marner F.J., Mynderse J. and Moore R.E. (1978) (-)-*Trans*-7(*S*)-methoxytetradec-4-enoic acid and related amides from the marine cyanophyte *Lyngbya majuscula*. *Phytochemistry*, 17: 2091-2095

Cardellina J.H., Marner F.J. and Moore R.E. (1979) Seaweed dermatitis: structure of lyngbyatoxin A. *Science*, 204: 193-5

Carlsson G. (1983) Validity of injury data collected by interview: a study of men born in 1914 and 1923. *J. Neurol. Neurosurg. Psychiatry*, 46: 818-823

Carmichael W. (2001) Health effects of toxin-producing cyanobacteria: "The CyanoHABs". *Hum. Ecol. Risk Assessment*, 7: 1393-1407

Carmichael W.W. (1986) Algal Toxins. In: *Advances in Botanical Research* (Ed. Callow J.). Academic Press, London, pp. 47-101

Carmichael W.W. (1994) The toxins of cyanobacteria. *Sci. Am.*, 270: 78-86

Carmichael W.W. (1997) The Cyanotoxins. In: *Advances in Botanical Research.* Academic Press, London, pp. 211-256

Carmichael W.W. and Falconer I.R. (1993) Diseases related to freshwater blue-green alga toxins and control measures. In: *Algal Toxins in Seafood and Drinking Water* (Ed. Falconer I.). Academic Press, London, pp. 187-210

Carmichael W.W., Eschedor J., Patterson G. and Moore R.E. (1988) Toxicity and partial structure of a hepatotoxic peptide produced by the cyanobacterium *Nodularia spumigena* Mertens emend. L575 from New Zealand. *Appl. Environ. Microbiol.*, 54: 2257-2263

Carmichael W.W., Evans W.R., Yin Q.Q., Bell P. and Moczydlowski E. (1997) Evidence for paralytic shellfish poisons in the freshwater cyanobacterium *Lyngbya wollei* (Farlow ex Gomont) comb. nov. *Appl. Environ. Microbiol.*, 63: 3104-3110

Carpenter E.J. (1983) Physiology and ecology of marine planktonic *Oscillatoria (Trichodesmium). Marine Biol. Lett.*, 4: 69-85

Carter D., Moore R.E., Mynderse J., Niemczura W. and Todd J. (1984) Structure of majusculamide C, a cyclic depsipeptide from *Lyngbya majuscula*. *J. Org. Chem.*, 49: 236-241

Cash W. and Moss A. (1972) Optimum recall period for reporting persons injured in motor vehicle accidents. *Data Evaluation Methods Res.*, 2: 1-33

Cheroske A., Williams S. and Carpenter R. (2000) Effects of physical and biological disturbances on algal turfs in Kaneohe Bay, Hawaii. *J. Exp. Mar. Biol. Ecol.*, 248: 1-34

Choi S. and Kim S. (1998) Lipopolysaccharide inhibition of rat hepatic microsomal epoxide hydrolase and glutathione S-transferase gene expression irrespective of nuclear factor-KB activation. *Biochem. Pharmacol.*, 56: 1427-1436

Chorus I., Falconer I., Salas H. and Bartrum J. (2000) Health risks caused by freshwater cyanobacteria in recreational waters. *J. Toxicol. Environ. Health*, 3: 323-347

Chu G. (1959) Seaweed dermatitis apparently caused by a marine alga. Laboratory observations. Proceedings of the 34th annual meeting of the Hawaiian academy of Sciences. pp. 19

Chung W.-Y., Jung Y.-J., Surh Y.-J., Lee S.-S. and Park K.-K. (2001) Antioxidative and antitumor promoting effects of [6]-paradol and its homologs. *Mutat. Res.*, 496: 199-206

Cichra M.F., Badylak S., Henderson N., Rueter B.H. and Philips E.J. (1995) Phytoplankton community structure in the open water zone of a shallow subtropical lake (Lake Okeechobee, Florida, USA). *Ergeb. Limnol.*, 45: 157-175

Clark M.W., McConchie D., Saenger P. and Pillsworth M. (1997) Hydrological controls on copper, cadmium, lead and zinc concentrations in an anthropogenically polluted mangrove ecosystem, Wynnum, Brisbane, Australia. *J. Coastal Res.*, 13: 1150-1158

Codd G.A., Bell S., Kunimitsu K., Ward C., Beattie K. and Metcalf J. (1999) Cyanobacterial toxins, exposure routes and human health. *Eur. J. Phycol.*, 34: 405-415

Codd G.A., Steffensen D.A., Burch M.D. and Baker P.D. (1994) Toxic blooms of cyanobacteria in Lake Alexandrina, South Australia - Learning from history. *Aust. J. Mar. Fresh. Res.*, 45; 731-736

Coenraads P., Bleumink E. and Nater J. (1975) Susceptibility to primary irritants. *Contact Dermatitis*, 1: 377-381

Collier D.N. (2002) Cutaneous infections from coastal and marine bacteria. *Dermatol Ther*, 15: 1-9

Collins M., Gowans C., Garro F., Estervif D. and Swanson T. (1981) Temporal association between an algal bloom and mutagenicity in a water reservoir. In: *The Water Environment, Algal Toxins and Health* (Ed. Carmichael WW). Plenum Press, New York, pp. 271-284

Cotovio J., Leclarie J. and Roguet R. (1997) Cytochrome P450-depentant enzyme activities in normal adult human keratinocytes and transformed human keratinocytes. *In Vitro Toxicology: Journal of Molecular and Cellular Toxicology*, 10: 207-216

Coughlin S. (1990) Recall bias in epidemiological studies. *J. Clin. Epidemiol.*, 43: 87-91

Cua A., Wilhelm K., Maibach H. and Wilson D. (1990) Cutaneous sodium lauryl sulfate irritation potenial: Age and regional variability. *Br. J. Dermatol.*, 123: 607-613

Czernieki B., Witz G., Reilly C. and Gad S. (1988) The development of contact hypersensitivity in mouse skin is suppressed by tumor promoters. *J. Appl. Toxicol.*, 8: 1-8

Dahlgren J., Warshaw R., Horsak R., Parker F. and Takhar H. (2003a) Exposure assessment of residents living near a wood treatment plant. *Environ. Res.*, 92: 99-109

Dahlgren J., Warshaw R., Thornton J., Anderson-Mahoney C. and Takhar H. (2003b) Health effects on nearby residents of a wood treatment plant. *Environ. Res.*, 92: 92-8

Dasgupta T., Banejee S., Yadav P. and Rao A. (2001) Chemomodulation of carcinogen metabolising enzymes, antioxidant profiles and skin forestomach papillogenesis by *Spirolena platensis*. *Mol. Cell. Biochem.*, 226: 27-38

Davie P. (1998) *Wild Guide to Moreton Bay*. Queensland Museum, Brisbane.

Dawson E., Aleem A. and Halstead B. (1955) Marine algae from Palmyra Islands with special reference to the feeding habits and toxicology of reef fishes. *Allan Hancock Publ. USC*, Occasional paper 17:

De Donno A., Privitera G., Fersini D., Conte E., Parisi E., Vitelli G. and Marra C. (1994) Effect on the number of bathers on microbial indicator densities in recreational seawater. *Ig. Mod.*

De Philippis R., Sili C., Faraloni C. and Vincenzini M. (2002) Occurrence and significance of exopolysaccharide-producing cyanobacteria in the benthic mucilaginous aggregates of the Tyrrhenian Sea (Tuscan Archipelago). *Ann. Microbiol.*, 52: 1-11

Dean J., Coulobier D., Smith D., Brendel K., Arner T. and Dean A. (1995) Epi Info: A word-processing, database and statistics program for public health on IBM-compatible microcomputers. Version 6. Atlanta, USA

DeLeo V., Taylor S., Belsito D., Fowler J., Fransway A., Maibach H., Marks J., Mathias C., Nethercott J., Pratt M., Reitschel R., Sherertz E., Storrs F. and Taylor J. (2002) The effect of race and ethinicity on patch test results. *J. Am. Acad. Dermatol.*, 46: S107-S112

DeMott W.W. and Moxter F. (1991) Foraging on cyanobacteria by copepods: Responses to chemical defences and resource abundance. *Ecology*, 72: 1820-1834

DeMott W.W., Zhang Q.-X. and Carmichael W.W. (1991) Effects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and three species of *Daphnia*. *Limnol. Oceanog.*, 36: 1346-1357

Dennison W.C. and Abal E. (1999) *Moreton Bay Study: A Scientific Basis for the Healthy Waterways Campaign*. South East Queensland Regional Water Quality Management, Brisbane.

Dennison W.C., O'Neil J.M., Duffy E., J., Oliver P.E. and Shaw G.R. (1999) Blooms of the cyanobacterium *Lyngbya majuscula* in coastal waters of Queensland, Australia. *Bulletin de l'Institut Oceanographique Monaco*, Special issue 19: 501-506.

DiGiulio R., Washburn P., Wenning R., Winston G. and Jewell C. (1989) Biochemical responses in aquatic animals: a review of determinants of oxidative stress. *Environ. Toxicol.*, 8: 1103-1123

Dow C. and Swoboda U. (2000) Cyanotoxins. In: *The Ecology of Cyanobacteria* (eds. Whitton B and Potts M). Kluwer Academic Publishers, Dordrecht, pp. 614-632

Dyer J., Forgie D., Martin B. and Martin D. (1992) Effects of selected copper(II)-chelate compounds on the rates of production of oxygen by filamentous algae. *Biomed. Lett.*, 47: 363-369

Edwards, C., Bettie, K., Scrimgeour, C. & Codd, G. (1992). Identification of anatoxin-a in benthic cyanobacteria (blue-green algae) and in associated dog poisoning at Loch Insh, Scotland. *Toxicon*, 30, 1165-1167

el Saadi O., Esterman Adrian J., Cameron S. and Roder David M. (1995) Murray River water, raised cyanobacterial cell counts, and gastrointestinal and dermatological symptoms. *Med. J. Aust.*, 162: 122-125

Elder G.H., Hunter P.R. and Codd G.A. (1993) Hazardous freshwater cyanobacteria (blue-green algae). *Lancet*, 341: 1519-1520

Eliasson L., Kallin B., Patarroyo M., Klein M., Fujiki H. and Sugimura T. (1983) Activation of the EBV-cycle and aggregation of human blood lymphocytes by tumour promoters teleocidin, lyngbyatoxin A, aplysiatoxin and debromoaplysiatoxin. *Int. J. Cancer*, 31: 7-11

Entzeroth M., Blackman A.J., Mynderse J.S. and Moore R.E. (1985) Structures and stereochemistries of oscillatoxin B, 31-noroscillatoxin B, oscillatoxin D and 30-methyloscillatoxin D. *J. Org. Chem.*, 50: 1255-1259

Epstein P. (1993) Algal blooms in the spread and persistence of cholera. *Biosystems*, 31: 209-221

Falch B., Koning G., Wright A., Sticher O., Angerhofer C., Pezzuto J. and Bachmann H. (1995) Biological activities of cyanobacteria: evaluation of extracts and pure compounds. *Planta Medica*, 61: 321-328

Falconer I. (1994) Health problems from exposure to cyanobacteria and proposed guidelines for drinking and recreational water. In: *Detection Methods for Cyanobacterial Toxins.* (eds. Codd G., Jefferies T., Keevil C. and Potter E.). Royal Society of Chemistry, Cambridge, UK, pp. 3-10

Falconer I., R., Burch M., D., Steffensen D.A., Choice M. and Coverdale O.R. (1994) Toxicity of the blue-green alga (Cyanobacterium) *Microcystis aeruginosa* in drinking water to growing pigs, as an animal model for human injury and risk assessment. *Environ. Toxicol. Water Quality*, 9: 131-139

Fastner J., Heinze R. and Chorus I. (1995) Miscrocystin-content hepatotoxicity and cytotoxicity of cyanobacteria in some German waterbodies. *Water Sci. Technol.*, 32: 165-170

Fastner J., Heinze R., Humpage A., Mischke U., Eaglesham G. and Chorus I. (2002) Cylindrospermopsin occurrence in two German lakes and preliminary assessment of toxicity and toxin production of *Cylindrospermopsis raciborskii* (Cyanobacteria) isolates. Toxicon 42: 313-321

Faulkner D.J. (1999) Marine natural products. *Nat. Prod. Rep.*, 16: 155-198

Faulkner D.J. (2000) Marine pharmacology. *Antonie Van Leeuwenhoek Int. J. Gen. Mol. Microbiol.*, 77: 135-145

Faust M., Altenberger R., Boedeker W. and Grimme L. (1994) Algal toxicity of binary combinations of pesticides. *Bull. Environ. Contam. Toxicol.*, 53: 134-141

Ferley J.P., Zmirou D., Balducci F., Baleux B., Fera P., Larbaigt G., Jacq E., Moissonnier B., Blineau A. and Boudot J. (1989) Epidemiological significance of microbiological pollution criteria for river recreational waters. *Int. J. Epidemiol.*, 18: 198-205

Filali-Mouhim R. and Hours M. (1995) [Les activities antivirales des polysaccharide sulfates]. *Acta Bot. Gall.*, 142: 125-130

Fisher A. (1995) Aquatic Dermatoses. In: *Fisher's Contact Dermatitis* (eds. Rietschel R. and Fowler J.). Williams and Wilkins, Baltimore, USA, pp. 920-963

Fisher P., Miranda A., Mufson A., Weinstein L., Fujiki H. and Weinstein I. (1982) Effects of teleocidin and the phorbol ester tumor promoters on cell transformation, differentiation and phospholipid metabolism. *Cancer Res.*, 42: 2829-2835

Fleisher J.M., Jones F., Kay D., Stanwell Smith R., Wyer M. and Morano R. (1993) Water and non-water-related risk factors for gastroenteritis among bathers exposed to sewage-contaminated marine waters. *Int. J. Epidemiol.*, 22: 698-708

Fleisher J.M., Kay D., Wyer M.D. and Godfree A.F. (1998) Estimates of the severity of illnesses associated with bathing in marine recreational waters contaminated with domestic sewage. *Int. J. Epidemiol.*, 27: 722-6

Fogg G.E., Stewart W.D.P., Fay P. and Walsby A.E. (1973) *The Blue-Green Algae.* Academic Press, London.

Forsyth D., James M. and Cryer M. (1990) Alteration of seasonal and diel patterns in vertical migration of zooplankton by *Anabaena* and planktivorous fish. *Arch. Hydrobiol.*, 117: 385-404

Francis G. (1878) Poisonous Australian lake. *Nature*, 18: 11-12

Frenk E., Mancarella A. and Vion B. (1990) Delayed skin reaction caused by a coelenterate. *Dermatologica*, 181: 241-2

Freudenthal A. (1991) Seabather's eruption: Range extended northward and a causative organism identified. *Revue Internationale d'Oceanographie Medicale*, 101: 137-147

Freudenthal A.R. and Joseph P.R. (1993) Seabather's eruption. *N. Engl. J. Med.*, 329: 542-4

Frosch, P., Duncan, S. and Kligman, A. (1980). Cutaneous biometrics I: The response of human skin to dimethyl sulphoxide. *Br.J. Dermatol.*, 102; 263-274.

Frosch, P. and Kligman, A. (1977). Rapid blister formation in human skin with ammonium hydroxide. *Br.J. Dermatol.*, 96; 461-473.

Frosch, P. and Wissing, C. (1982). Cutaneous sensitivity to ultraviolet light and chemical irritants. *Arch. Dermatol. Res.*, 272; 269-278.

Fujiki H., Ikegami K., Hakii H., Suganuma M., Yamaizumi Z., Yamazato K., Moore R.E. and Sugimura T. (1985) A blue-green alga from Okinawa contains aplysiatoxins, the third class of tumor promoters. *Jpn. J. Cancer Res.*, 76: 257-9

Fujiki H., Mori M., Nakayasu M., Terada M., Sugimura T. and Moore R.E. (1981) Indole alkaloids: dihydroteleocidin B, teleocidin, and lyngbyatoxin A as members of a new class of tumor promoters. *Proc. Natl. Acad. Sci. U. S. A.*, 78: 3872-6

Fujiki H., Suganuma M. and Nakayasu M. (1982) The third class of tumor promoters, polyacetates (debromoaplysiatoxins and aplysiatoxin), can differentiate biological actions relevant to tumor promoters. *Gann*, 73: 495-497

Fujiki H., Suganuma M., Hakii H., Bartolini G., Moore R.E., Takayama S. and Sugimura T. (1984a) A two-stage mouse skin carcinogenesis study of lyngbyatoxin A. *J. Cancer Res. Clin. Oncol.*, 108: 174-6

Fujiki H., Suganuma M., Matsukura N., Sugimura T. and Takayama S. (1982b) Teleocidin from *Streptomyces* is a potent promoter of mouse skin carcinogenesis. *Carcinogenesis*, 3: 895-898

Fujiki H., Suganuma M., Suguri H., Yoshizawa S., Takagi K., Nakayasu M., Ojika M., Yamada K., Yasumoto T., Moore R.E. and Sugimura T. (1990) New

Tumor Promoters from Marine Natural Products. In: *Marine Toxins: Origin, Structure and Molecular Pharmacology* (eds. Hall S and Strichartz G). American Chemical Society pp. 232-240

Fujiki H., Suganuma M., Tahira T., Yoshioka A., Nakayasu M., Endo Y., Shudo K., Takayama S., Moore R.E. and Sugimura T. (1983a) Nakahara memorial lecture. New classes of tumor promoters: teleocidin, aplysiatoxin, and palytoxin. *Princess Takamatsu Symp*, 14: 37-45

Fujiki H., Sugimura T. and Moore R.E. (1983b) New classes of environmental tumor promoters: indole alkaloids and polyacetates. *Environ. Health Perspect.*, 50: 85-90

Fujiki H., Takagi K., Miyake R., Kikkawa U., Nishizuka Y. & Sugimura T. (1984b) Activation of calcium-activated, phospholipid-dependent protein kinase (protein kinase C) by new classes of tumor promoters: teleocidin and debromoaplysiatoxin. *Biochem. Biophys. Res. Commun.*, 120: 339-343

Gad S. (1994) The mouse ear swelling test (MEST) in the 1990s. *Toxicology*, 93: 33-46

Gad S., Dunn B., Dobbs D., Reilly C. and Walsh R. (1986) Development and validation of an alternative dermal sensitisation test: the mouse ear swelling test (MEST). *Toxicol. Appl. Pharmacol.*, 84: 93-114

Gallimore W. and Scheur P. (2000) Malyngamides O and P from the sea hare *Stylocheilus longicauda*. *J. Nat. Prod.*, 63: 1422-1424

Gallon J., Kittakoop O. and Brown E. (1994) Biosynthesis of anatoxin-a by *Anabaena flos-aquae*: examination of primary enzymic steps. *Phytochemistry*, 35: 1195-1203

Garbacki N., Gloagguen V., Damas J., Hoffmann L., Tits M. and Angenot L. (2000) Inhibition of croton oil-induced oedema in mice ear skin by capsular polysaccarides from cyanobacteria. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 361: 460-464

Gaveriaux C., Fehr T., Montecino Rodriguez E., Sanglier J.J. and Loor F. (1988) Protein kinase C activators of the teleocidin family decrease the IgE-binding capacity of rat basophilic leukemia cells. *Int Arch Allergy Appl Immunol*, 86: 465-71

Gelardi A., Morini F., Dusatti F., Penco S. and Ferro M. (2001) Induction by xenobiotics of phase I and phase II enzyme activities in the human keratinocyte cell line NCTC 2544. *Toxicol. in Vitro*, 15: 701-711

Gerwick W.H., Proteau P.J., Nagle D.G., Hamel E., Blokhin A. and Slate D.L. (1994) Structure of a curacin A, a novel antimitotic, antiproliferative, and brine shrimp toxic natural product from the marine cyanobacterium *Lyngbya majuscula*. *J. Org. Chem.*, 59: 1243-1245

Gerwick W.H., Reyes S. and Alvarado B. (1987) Two malyngamides from the Caribbean cyanobacterium *Lyngbya majuscula*. *Phytochemistry*, 26: 1701-1704

Goldstein B., Witz G., Amoruso M., Stone D. and Troll W. (1981) Stimulation of human polymorphonuclear leukocyte superoxide anion radical production by tumour promoters. *Cancer Lett.*, 11: 257-262

Gonzalez E. (1989) Schistosomiasis, cercarial dermatitis, and marine dermatitis. *Dermatol. Clin.*, 7: 291-300

Gonzalez J. and Suttle C. (1993) Grazing by marine nanoflagellates on viruses and virus-shaped particles: ingestion and digestion. *Marine Ecol. Prog. Series*, 94: 1-10

Gorham P.R. and Carmichael W.W. (1988) Hazards of freshwater blue-greens (cyanobacteria). In: *Algae in Human Affairs* (eds. Lembi CA and Waaland JR). Cambridge University Press, New York, pp. 403-431

Graber M.A. and Gerwick W.H. (1998) Kalkipyrone, a toxic gamma-pyrone from an assemblage of the marine cyanobacteria *Lyngbya majuscula* and *Tolypothrix* sp. *J. Nat. Prod.*, 61: 677-80

Grauer F. (1959a) Dermatitis eschorotica caused by marine alga. *Hawaii Med. J.*, 19: 32-36

Grauer F. (1959b) Seaweed dermatitis apparently caused by a marine alga. Clinical investigative procedures. Proceedings of the 34th annual meeting of the Hawaiian Academy of Science. pp. 18

Grauer F. and Arnold H. (1961) Seaweed dermatitis: first report of dermatitis-producing marine algae. *Arch. Dermatol.*, 84: 720-732

Gross E., D. and Martin Dean F. (1996) Iron dependence of *Lyngbya majuscula*. *J. Aquatic Plant Man.*, 34: 17-20

Grove G., Duncan S. and Kligman A. (1982) Effect of aging on the blistering of human skin with ammonium hydroxide. *Br. J. Dermatol.*, 107: 393-400

Gupta N., Pant S., Vijayaraghavan R. and Rao P (2003) Comparative toxicity evaluation of cyanobacterial cyclic peptide toxin microcystin variants (LR, RR, YR) in mice. Toxicology 188: 285-296

Habekost R.C., Fraser I.M. and Halstead B.W. (1955) Toxicology - Observations on toxic marine algae. *J. Washington Acad. Sci.*, 45: 101

Haile R., Witte J., Gold M., Cressey R., McGee C., Millikan R., Glasser A., Harawa N., Ervin C., Harmon P., Harper J., Dermand J., Alamillo J., Barret K., Nides M. and Wang G.-y. (1999) The health effects of swimming in ocean water contaminated by storm drain runoff. *Epidemiology*, 10: 355-363

Halstead B. (1987) Coelenterate (Cnidarian) stings and wounds. *Clin. Dermatol.*, 5: 8-13

Harel Y., Overpeck M. and Jones D. (1994) The effects of recall on estimating annual nonfatal injury rates for children and adolescents. *Am. J. Public Health*, 84: 599-605

Harrigan G.G., Yoshida W.Y., Moore R.E., Nagle D.G., Park P.U., Biggs J., Paul V.J., Mooberry S.L., Corbett T.H. and Valeriote F.A. (1998) Isolation, structure determination, and biological activity of dolastatin 12 and lyngbyastatin I from *Lyngbya majuscula/Schizothrix calcicola* cyanobacterial assemblages. *Nat. Prod. Rep.*, 61: 1221-1225

Harrison R., Holt D. and Elton P. (2002) Do postage-stamps increase response rates to postal surveys? A randomized controlled trial. *Int. J. Epidemiol.*, 31: 872-874

Harvell C.D., Kim K., Burkholder J.M., Colwell R.R., Epstein P.R., Grimes D.J., Hofmann E.E., Lipp E.K., Osterhaus A., Overstreet R.M., Porter J.W., Smith G.W. and Vasta G.R. (1999) Review: Marine ecology - Emerging marine diseases - Climate links and anthropogenic factors. *Science*, 285: 1505-1510

Hashimoto Y. (1979) *Marine Toxins and Other Bioactive Marine Metabolites*. Japan Scientific Societies Press, Tokyo.

Hashimoto Y., Kamiy H., Yamazato K. and Nozawa K. (1976) Occurrence of toxic blue-green alga inducing skin dermatitis in Okinawa. In: *Animal, Plant and*

Microbial Toxins (eds. Ohsaka A, Hayashi K and Sawai Y). Plenum Press, New York, pp. 333-338

Havell C., Kim K., Burkholder J., Colwell R., Epstien P., Grimes D., Hofmann E., Lipp E., Osterhaus A., Overstreet R., Porter J., Smith G. and Vasta G. (1999) Review - marine ecology - emerging marine diseases - climate links and anthropogenic factors. *Science*, 285: 1505-1510

Havens K., E., Phlips Edward J., Cichra Mary F. and Li Bai L. (1998) Light availability as a possible regulator of cyanobacteria species composition in a shallow subtropical lake. *Freshwater Biol.*, 39: 547-556

Hawkins P., Runnegar M., Jackson A. and Falconer I. (1985) Severe hepatotoxicity caused by the tropical cyanobacterium (blue-green alga) *Cylindrospermopsis rackiborskii* (Woloszynska) Seenaya and Subba Raju isolated from a domestic water supply reservoir. *Appl. Environ. Microbiol.*, 50: 1292-1295

Hawser S., Codd G., Capone D. and Carpenter E. (1991) A neurotoxic factor associated with the bloom-forming cyanobacterium *Trichodesmium*. *Toxicon*, 29: 277-278

Hayes B. and Meade B. (1999) Contact sensitivity to selected acrylate compounds in B6C3F1 mice: relative potency, cross reactivity, and comparison of test methods. *Drug Chem. Toxicol.*, 22: 491-506

Hayes B., Gerber P., Griffey S. and Meade B. (1998) Contact hypersensitivity to dicyclohexylcarbodiimide and diisopropylcarbodiimide in female B6C3F1 mice. *Drug Chem. Toxicol.*, 21: 195-206

Hecker E. (1971) Isolation and characterization of the cocarcinogenic principles from croton oil. In: *Methods in Cancer Research* (Ed. Busch H). Academic Press, New York-London, pp. 439-484

Heil C., O'Donohue M., Miller C. and Dennison W. (1998) Phytoplankton community response to a flood event. In: *Moreton Bay and Catchment* (eds. Tibbets I, Hall N and Dennison W). School of Marine Science, University of Queensland, Brisbane, pp. 569-584

Heise (1949) Symptoms of hayfever caused by algae. *J. Allergy*, 20: 383-385

Helfrich P. and Banner A. (1960) Hallucinatory mullet poisoning. *J. Trop. Med. Hyg.*, 63: 86-89

Henrickson S., Wong T., Allen P., Ford T. and Epstein P. (2001) Marine swimming-related illness: implications for monitoring and environmental policy. *Environ. Health Perspect.*, 109: 645-650

Heredia-Tapia, A., Arredondo-Vega, B., Nunez-Vazquez, E., Yasumoto, T., Yasuda, M. & Ochoa, J. (2002). Isolation of *Prorocentrum lima* (Syn. *Exuviaella lima*) and diarrhetic shellfish poisoning (DSP) risk assessment in the Gulf of California, Mexico. *Toxicon*, 40, 1121-1127

Hernandez M., Robinson I., Aguilar A., Gonzalez L.M., LopezJurado L.F., Reyero M.I., Cacho E., Franco J., LopezRodas V. and Costas E. (1998) Did algal toxins cause monk seal mortality? *Nature*, 393: 28-29

Hertz-Picciotto I. (1995) Epidemiology and quantitative risk assessment: a bridge from science to policy. *Am. J. Public Health* 85(5):484-491

Hoffmann L. (1999) Marine cyanobacteria in tropical regions: diversity and ecology. *Eur. J. Phycol.*, 34: 371-379

Hoffmann L. and Demoulin V. (1991) Marine Cyanophyceae of Papua New Guinea: II. *Lyngbya bouillonii*, new species, a remarkable tropical reef-inhabiting blue-green alga. *Belg. J. Bot.*, 124: 82-88

Holzer P. (1988) Local effector functions of capsaicin-sensitive sensory nerve endings: Involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. *Neuroscience*, 24: 739-768

Hooper G.J., Orjala J., Schatzman R.C. and Gerwick W.H. (1998) Carmabins A and B, new lipopeptides from the Caribbean cyanobacterium *Lyngbya majuscula*. *J. Nat. Prod.*, 61: 529-33

Horowitz A., Fujiki H. and Weinstein I. (1983) Comparative effects of aplysiatoxin, dibromoaplysiatoxin, and teleocidin on receptor binding and phospholipid metabolism. *Cancer Res.*, 43: 1529-1539

Howell M., Manetz T. and Meade B. (2000) Comparison of murine assays for the identification of chemical sensitizers. *Toxicol. Meth.*, 10: 1-15

Hsiao W.L., Gattoni Celli S. and Weinstein I.B. (1984) Oncogene-induced transformation of C3H 10T1/2 cells is enhanced by tumor promoters. *Science*, 226: 552-5

Hsiao W.L., Wu T. and Weinstein I.B. (1986) Oncogene-induced transformation of a rat embryo fibroblast cell line is enhanced by tumor promoters. *Mol. Cell. Biol.*, 6: 1943-50

Hunter P.R. (1994) An epidemiological critique of reports of human illness associated with cyanobacteria. In: *Detection Methods for Cyanobacterial Toxins* (eds. Codd G, Jefferies T, Keevil C and Potter E). The Royal Society of Chemistry, Cambridge,

Hunter P.R. (1998) Cyanobacterial toxins and human health. *J. Appl. Microbiol. (supp.)*, 84: 35S-40S

Hurd C. (2000) Water motion, marine macroalgal physiology, and production. *J. Phycol.*, 36: 453-472

Inoue H., Asaka T., Nagata N. and Koshihara Y. (1997) Mechanism of mustard oil-induced skin inflammation in mice. *Eur. J. Pharmacol.*, 333: 231-240

Ito E. and Nagai H. (1998) Morphological observations of diarrhea in mice caused by aplysiatoxin, the causative agent of the red alga *Gracilaria coronopifolia* poisoning in Hawaii. *Toxicon*, 36: 1913-1920

Ito E. and Nagai H. (2000) Bleeding from the small intestine casued by aplysiatoxin, the causative agent of the red algae *Gracilaria coronopifolia* poisoning in Hawaii. *Toxicon*, 38: 123-132

Ito E., Satake M. and Yasumoto T. (2002) Pathological effects of lyngbyatoxin A upon mice. *Toxicon*, 40: 551-556

Izumi A.K. and Moore R.E. (1987) Seaweed (*Lyngbya majuscula*) dermatitis. *Clin. Dermatol.*, 5: 92-100

Jackson W., Carmicheal W. and Carpenter E. (2001) The paralytic shellfish toxins, n-sulfocarbamoyl and gonyautaxin, are the major neurotoxic factors associated with bloom-forming *Trichodesmium* spp. Fifth International Conference on Toxic Cyanobacteria Noosa, Australia pp.

Jiang S. and Paul J. (1998) Gene transfer by gene induction in the marine environment. *Appl. Environ. Microbiol.*, 64: 2780-2787

Jimenez J. and Scheuer P. (2001) New lipopeptides from the Caribbean cyanobacterium *Lyngbya majuscula*. *J. Nat. Prod.*, 64: 200-203

Jones G. and Sivonen K. (1999) Cyanobacterial Toxins. In: *Toxic Cyanobacteria in Water* (eds. Chorus I. and Bartrum J.) E and FN Spon, London, pp. 41-111

Jungmann D. (1992) Toxic compounds isolated from *Microcystis* PCC78806 that are more active against *Daphnia* than two microcystins. *Limnol. Oceanog.*, 37: 1777-1783

Jungmann D. and Benndorf J. (1994) Toxicity to *Daphnia* of a compound extracted from laboratory and natural *Microcystis* spp., and the role of microcystins. *Freshwater Biol.*, 32: 13-20

Kadyk D., McCarter K., Achen F. and Belsito D. (2003) Quality of life in patients with allergic contact dermatitis. *J Am Acad Dermatol.*, 49: 1037-1048

Kaebernick M. and Nielan B. (2001) Ecological and molecular investigations of cyanotoxin production. *FEMS Microbiol. Ecol.*, 35: 1-9

Kan Y., Fujita T., Nagai H., Sakamoto B. and Hokama Y. (1998) Malyngamides M and N from the Hawaiian red alga *Gracilaria coronopifolia. J. Nat. Prod.*, 61: 152-155

Kan Y., Sakamoto B., Fujita T. and Nagai H. (2000) New malyngamides from the Hawaiian cyanobacterium *Lyngbya majuscula. J. Nat. Prod.*, 63: 1599-1602

Kao C. (1993) Paralytic shellfish poisoning. In: *Algal toxins in seafood and drinking water* (Ed. Falconer I). Academic Press, London, pp. 75-86

Kato Y. and Scheuer P.J. (1974) Aplysiatoxin and debromoaplysiatoxin, constituents of the marine mollusk *Stylocheilus longicauda* (Quoy and Gaimard, 1824). *J. Am. Chem. Soc.*, 96: 2245-6

Kato Y. and Scheuer P.J. (1975) The aplysiatoxins. *Pure Appl. Chem.*, 41: 1-14

Keisari Y., Geva I., Flescher E., Goldin H. and Lavie G. (1985) Stimulation of human monocyte oxidative burst and related cytotoxicity by tumor-promoting and non-tumor-promoting diterpene esters, indole alkaloids and polyacetate-type agents. *Int. J. Cancer*, 36: 467-472

Keleti G. and Sykora. J.L. (1982) Production and properties of cyanobacterial endotoxins. *Appl. Environ. Microbiol.*, 43: 104-109

Kirpenko Y.A., Sirenko J.L. and Kirpenko N.L. (1981) Some aspects concerning remote after-effects of blue-green algal toxin impact on warm-blooded animals. In: *The Water Environment, Algal Toxins and Health* (Ed. Carmichael WW). Plenum Press, New York, pp. 257-269

Klimuk S., Semple S., Nahirney P., Mullen M., Bennett C., Scherrer P. and Hope M. (2000) Enhanced anti-inflammatory activity of a liposomal intercellular adhesion molecule-1 antisense oligodeoxynucleotide in an acute model of contact hypersensitivity. *J. Pharmacol. Exp. Ther.*, 292: 480-488

Koehn F.E., Longley R.E. and Reed J.K. (1992) Microcolins A and B, new immunosuppressive peptides from the blue-green alga *Lyngbya majuscula. J. Nat. Prod.*, 55: 613-619

Kokelj F., Del Negro P. and Tubaro A. (1989) [Dermatotoxicity caused by *Chrysaora hysoscella.* Presentation of a case]. *G. Ital. Dermatol. Venereol.*, 124: 297-8

Kokelj F., Mianzan H., Avian M. and Burnett J.W. (1993) Dermatitis due to *Olindias sambaquiensis*: a case report. *Cutis*, 51: 339-42

Koop K., Booth D., Broadbent A., Brodie J., Bucher D., Capone D., Coll J., Dennison W., Erdmann M., Harrison P., Hoegh-Guldberg O., Hutchings P., Jones G., Larkum A., O'Neil J., Steven A., Tentori E., Ward S., Williamson J. and Yellowlees D. (2001) ENCORE: The effect of nutrient enrichment on coral reefs, synthesis of results and conclusions. *Mar Pollut. Bull.*, 42: 91-120

Korkeila K., Suominen S., Ahvenainen J., Ojanlatva A., Rautava P., Helenius H. and Koskenvuo M. (2001) Non-response and related factors in a nation-wide health survey. *Eur. J. Epidemiol.*, 17: 991-999

Koyuncu H., Berkarda B., Baykut F., Soybir G., Alatli C., Gul H. and Altun M. (1999) Preventive effect of hesperidin againt inflammation in CD-1 mouse skin caused by tumor promoter. *Anticancer Res.*, 19: 3237-3242

Kozikowski A.P., Shum P.W., Basu A. and Lazo J.S. (1991) Synthesis of structural analogues of lyngbyatoxin A and their evaluation as activators of protein kinase C. *J. Med. Chem.*, 34: 2420-30

Kuffner I. and Paul V. (2001) Effects of nitrate, phosphate and iron on the growth of macroalgae and benthic cyanobacteria from Cocos Lagoon, Guam. *Marine Ecol. Prog. Series*, 222: 63-72

Kuiper-Goodman T., Falconer I. and Fitzgerald J. (1999) Human Health Aspects. In: *Toxic Cyanobacteria in Water: a Guide to their Public Health Consequences, Monitoring and Management.* (eds. Chorus I and Bartrum J). E and FN Spon, London, pp. 113-153

Kuiper-Goodman T., Falconer I. and Fitzgerald J. (1999) Human Health Aspects. In: *Toxic Cyanobacteria in Water* (eds. Chorus I. and Bartrum J.). E and FN Spon, London, pp. 113-153

Kullavanijaya P., Sirimachan S. and Bhuddhavudhikrai P. (1993) *Mycobacterium marinum* cutaneous infections acquired from occupations and hobbies. *Int. J. Dermatol.*, 32: 504-7

Kyselkova I. and Marsalek B. (2000) Using *Daphnia pulex*, *Artemia salina* and *Tubifex tubifex* for cyanobacterial microcystins toxicity detection. *Biol. Brat.*, 55: 637-643

Lachowicz A. and Rebas E. (2002) Gender differences in steroid modulation of angiotensin II-induced protein kinase C activity in anterior pituitary of the rat. *Biochem Biophys Res Commun*, 294: 95-100

Lahti K., Rapala J., Färdig M., Niemelä M. and Sivonen K. (1997) Persistence of cyanobacterial hepatotoxin microcystin-LR, in particulate material and dissolved in lake water. *Water Res.*, 31: 1005-1012

Lam A., Prepas E., Spink D. and Hrudey S. (1995) Chemical control of hepatotoxic phytoplankton blooms: implications for human health. *Water Res.*, 29: 1845-1854

Lambertus M.W. and Mathisen G.E. (1988) *Mycobacterium marinum* infection in a patient with cryptosporidiosis and the acquired immunodeficiency syndrome. *Cutis*, 42: 38-40

Lammintausta K. and Kalimo K. (1981) Atopy and hand dermatitis in hospital wet work. *Contact Dermatitis*, 7: 301-308

Lammintausta K., Maibach H. and Wilson D. (1987) Irritant reactivity in males and females. *Contact Dermatitis*, 17: 276-280

Landen D.D. and Hendricks S. (1995) Effect of recall on reporting at-work injuries. *Public Health Rep.*, 110: 350-354

Landsberg J., Balazas G., Steidinger K., Baden D., Work T. and Russell D. (1999) The potential role of natural tumor promoters in marine turtle fibropapillomatosis. *J. Aquatic Anim. Health*, 11: 199-210

Lantinga H., Nater J. and Coenraads P. (1984) Prevalence, incidence and course of eczema on the hands and forearms in a sample of the general population. *Contact Dermatitis*, 10: 135-139

Lawrence J., Menard C. and Cleroux C. (1995) Evaluation of Prechromatographic Oxidation for Liquid Chromatographic Determination of Paralytic Shellfish Poisons in Shellfish. *Journal of the AOAC International*, 78: 514-520

Lehmann D., Tennant M.T., Silva D.T., McAullay D., Lannigan F., Coates H. and Stanley F.J. (2003) Benefits of swimming pools in two remote Aboriginal communities in Western Australia: intervention study. *Br. Med. J.*, 327: 415-419

Lejman E., Stoudmayer T., Grove G. and Kigman A. (1984) Age differences in poison ivy dermatitis. *Contact Dermatitis*, 11: 163-167

Leveque J. (1989) Measurement of transepidermal water loss. In: *Cutaneous Investigation in Health and Disease: Noninvasive Methods and Instrumentation* (Ed. Leveque J.). Dekker, New York, pp. 135-152

Lightner D. (1978) Possible toxic effects of the marine blue-green alga, *Spirulina subsalsa*, on the blue shrimp, *Panaesus stylirostis*. *J Invert Pathol.*, 32: 139-150

Loffler H., Hoffmann R., Happle R. and Effendy I. (2001) Murine auricular transepidermal water loss - a novel approach for evaluating irritant skin reaction in mice. *Clin. Exp. Dermatol.*, 26: 196-200

Long B., Jones G. and Orr P. (2001) Cellular microcystin content in N-limited *Microcystis aeruginosa* can be predicted from growth rate. *Appl. Environ. Microbiol.*, 67: 278-283

Luesch H., Pangilinan R., Yoshida W., Moore R.E. and Paul V. (2001a) Pitipeptolides A and B, New Cyclodepsipeptides from marine cyanobacterium *Lyngbya majuscula*. *J. Nat. Prod.*, 64: 304-307

Luesch H., Williams P., Yoshida W.Y., Moore R.E. and Paul Valerie J. (2002a) Ulongamides A-F, New beta-amino acid-containing cyclodepsipeptides from Palauan collections of the marine cyanobacteria *Lyngbya* sp. *J. Nat. Prod.*, 65: 996-1000

Luesch H., Yoshida W., Moore R.E. and Paul V. (2000a) Apramides A-G, novel lipopeptides from the marine cyanobacterium *Lyngbya majuscula*. *J. Nat. Prod.*, 63: 1106-1112

Luesch H., Yoshida W., Moore R.E. and Paul V. (2000b) Isolation and structure of the cytotoxin lyngbyabellin B and absolute configuration of lyngbyapeptin A from the marine cyanobacterium *Lyngbya majuscula*. *J. Nat. Prod.*, 63: 1437-1439

Luesch H., Yoshida W., Moore R.E. and Paul V. (2002b) New apratoxins of marine cyanobacterial origin from Guam and Palau. *Bioorg. Medicinal Med.*, 10: 1973-1978

Luesch H., Yoshida W.Y., Moore R.E. and Paul V.J. (1999) Lyngbyastatin 2 and norlyngbyastatin 2, analogues of dolastatin G and nordolastatin G from the marine cyanobacterium *Lyngbya majuscula*. *J. Nat. Prod.*, 62: 1702-1706

Luesch H., Yoshida W.Y., Moore R.E., Paul V.J. and Mooberry S.L. (2000c) Isolation, structure determination, and biological activity of lyngbyabellin A from the marine cyanobacterium Lyngbya majuscula. *J. Nat. Prod.*, 63: 611-615

Luesch H., Yoshida W.Y., Moore R.E., Paul Valerie J. and Corbett T.H. (2001b) Total structure determination of apratoxin A, a potent novel cytotoxin from the marine cyanobacterium *Lyngbya majuscula*. *J. Am. Chem. Soc.*, 123: 5418-5423

Lukac M. and Aegerter R. (1993) Influence of trace metals on growth and toxin production of *Microcystis aeruginosa*. *Toxicon*, 31: 293- 305

Lyck S., Gjolme N. and Utkilen H. (1996) Iron starvation increases toxicity of *Microcystis aeruginosa* CYA 228/1 (Chroococcales, Cyanophyceae). *Phycologia*, 35: 120-124

MacMillian J. and Molinski T. (2002) Caylobolide A, a unique 36-membered macrolactone from a Bahamian *Lyngbya majuscula*. *Organic Letters*, 4: 1535-1538

Macsween R., M. and Williams H.C. (1996) Seabather's eruption. A case of Caribbean itch. *Br. Med. J.*, 312: 957-958

Mahmood N. and Carmichael W.W. (1986a) Paralytic shellfish poisons produced by the freshwater cyanobacterium *Aphanizomenon flos-aquae* nh-5. *Toxicon*, 24: 175-186

Mahmood N. and Carmichael W.W. (1986b) The pharmacology of anataoxin-a(S), a neurotoxin produced by the freshwater cyanobacterium *Anabaena flos-aquae* NRC 525-17. *Toxicon*, 24: 425-434

Malten K., Fregert S., Bandmann H. and et al. (1971) Occupational dermatitis in five European dermatological departments. *Berufsdermatosen.*, 19: 1-13

Mandojana R. and Sims J. (1987) Miscellaneous dermatoses associated with the aquatic environment. *Clin. Dermatol.*, 5: 134-145

Manowitz N.R. and Rosenthal R.R. (1979) Cutaneous-systemic reactions to toxins and venoms of common marine organisms. *Cutis*, 23: 450-4

Marionnet D., Chambras C., Taysse L., C B. and Descheaux P. (1998) Modulation of drug-metabolising systems by bacterial endotoxin in carp liver and immune organs. *Ecotoxicol. Environ. Saf.*, 41: 189-194

Marner F.-J., Moore R.E., Hirotsu K. and Clardy J. (1977) Majusculamides A and B, two epimeric lipodipeptides from *Lyngbya majuscula* Gomont. *J. Org. Chem.*, 42: 2815-2819

Marquez B., Verdier Pinard P., Hamel E. and Gerwick W.H. (1998) Curacin D, an antimitotic agent from the marine cyanobacterium *Lyngbya majuscula*. *Phytochemistry*, 49: 2387-2389

Marshall K.L. and Vogt R.L. (1998) Illness associated with eating seaweed, Hawaii, 1994. *West. J. Med.*, 169: 293-5

Massey J. and Gonzalez J. (1976) Optimum recall periods for estimating accidental injuries in the National Health Interview Survey. Proceeding of the Annual General Meeting of the American Statistical Association. pp. 584-588

MBWCP (2002) Preliminary *Lyngbya* Management Strategy. March 2002 3.0. Moreton Bay Waterways and Catchments Partnership, Brisbane.

McPhail K. & Gerwick William H. (2003) Three new malyngamides from a Papua New Guinea collection of the marine cyanobacterium *Lyngbya majuscula*. *J. Nat. Prod.*, 66: 132-135

Mebs D. (2001) Toxicity in animals. Trends in evolution? *Toxicon*, 39: 87-96

Mesguiche V., Valls R., Piovetti L. and Peiffer G. (1999) Characterization and synthesis of (-)-7-methoxydodec-4(E)-enoic acid, a novel fatty acid isolated from *Lyngbya majuscula*. *Tetrahedron Lett.*, 40: 7473-7476

Mez, K., Hanselmann, J., Naegli, H. & Preisig, H. (1996). Protein-phosphatase inhibiting activity in cyanobacteria from alpine pastures in Switzerland. *Phycologia*, 35(suppl.), 133-139

Microsoft (1997a) Microsoft Access. 97 SR-2 Seattle, USA

Microsoft (1997b) Microsoft Excel. Version 97 SR-2 Seattle, USA

Milligan K., Marquez B., Williamson R., Davies-Cloeman M. and Gerwick W. (2000a) Two new malyngamides from a Madagascan *Lyngbya majuscula. J. Nat. Prod.*, 63: 965-968

Milligan K., Marquez B., Williamson R., and Gerwick W. (2000b) Lyngbyabellin B, a toxic and antifungal secondary metabolite from the marine cyanobacterium *Lyngbya majuscula. J. Nat. Prod., 63:* 1440-1443

Mirlean N., Vanz A. and Baisch P. (1999) Copper partition from sediments to water as determined by suspension experiments. 5th International Conference On Biogeochemistry of Trace Elements Wien, Austria pp. 488-489

Mitchell S., Faulkner D., Rubins K. and Bushman F. (2000) Dolastain 3 and two novel cyclic peptides from a Palauan collection of *Lyngbya majuscula. J. Nat Prod.*, 63: 279-282

Mittal A., Agarwal M.K. and Shivpuri D.N. (1979) Respiratory allergy to algae: clinical aspects. *Ann. Allergy*, 42: 253-6

Mock C., Acheampong F., Adjei S. and Koepsell T. (1999) The effect of recall on estimation of incidence rates for injury in Ghana. *Int. J. Epidemiol.*, 28: 750-755

Moikeha S.N. and Chu G.W. (1971a) Dermatitis-producing alga *Lyngbya majuscula* Gomont in Hawaii. II. Biological properties of the toxic factor. *J. Phycol.*, 7: 8-13

Moikeha S.N., Chu G.W. and Berger L.R. (1971b) Dermatitis-producing alga *Lyngbya majuscula* Gomont in Hawaii. I. Isolation and chemical characterisation of the toxic factor. *J. Phycol.*, 7: 4-8

Moore R.E. (1981) Toxins from marine blue-green algae. In: *The Water Environment - Algal Toxins and Health* (Ed. Carmichael WW). Plenum Press, New York, pp. 15-24

Moore R.E. (1984) Public Health and Toxins from Marine Blue-Green Algae. In: *Seafood Toxins* (Ed. Ragelis EP). American Chemical Society, Washington,

Moore R.E. (1996) Cyclic peptides and depsipeptides from cyanobacteria: A review. *J. Ind. Microbiol.*, 16: 134-143

Moore R.E. and Entzeroth M. (1988) Majusculamide D and deoxymajusculamide D, two cytotoxins from *Lyngbya majuscula. Phytochemistry*, 27: 3101-3104

Moore R.E., Blackman A., Cheuk C., Mynderse J., Matsumoto G., Clardy J., Woodard R. and Craig J. (1984) Absolute stereochemistries of the aplysiatoxins and oscillatoxin A. *J. Org. Chem.*, 49: 2484-2489

Moore R.E., Patterson G.M.L., Entzeroth M., Morimoto H., Suganuma M., Hakii H., Fujiki H. and Sugimura T. (1986) Binding studies of tritiated lyngbyatoxin A and tritiated debromoaplysiatoxin to the phorbol ester receptor in a mouse epidermal particulate fraction. *Carcinogenesis*, 7: 641-644

Mullin J.M., McGinn M.T., Snock K.V. and Imaizumi S. (1990) The effects of teleocidin and aplysiatoxin tumor promoters on epithelial tight junctions and transepithelial permeability: Comparison to phorbol esters. *Carcinogenesis*, 11: 377-386

Mur L., Skulberg O. and Utkilen H. (1999) Cyanobacteria in the environment. In: *Toxic Cyanobacteria in Water* (Eds. Chorus I and Bartrum J). WHO, London, pp. 15-40

Mynderse J.S. and Moore R.E. (1978) Malyngamides D and E, two trans-7-methoxy-9-methylhexadec-4-enamides from a deep water variety of the marine cyanophyte *Lyngbya majuscula*. *J. Org. Chem.*, 43: 4359-4363

Mynderse J.S., Moore R.E., Kashiwagi M. and Norton T.R. (1977) Antileukemia activity in the Osillatoriaceae: isolation of debromoaplysiatoxin from *Lyngbya*. *Science*, 196: 538-40

Nadai M., Sekido T., Matsuda I., Li I., Kitaichi K., Itoh A., Nabeshima T. and Hasegawa T. (1998) Time-dependant effects of *Klebsiella pneumoniae* endotoxin on hepatic drug metabolizing enzyme activity in rats. *J Pharmacol.*, 50: 871-879

Nagai H., Yasumoto T. and Hokama Y. (1996) Aplysiatoxin and debromoaplysiatoxin as the causative agents of a red alga *Gracilaria* coronopifolia poisoning in Hawaii. *Toxicon*, 34: 753-761

Nagai H., Yasumoto T. and Hokama Y. (1997) Manauealides, some of the causative agents of a red alga *Gracilaria coronopifolia* poisoning in Hawaii. *J. Nat. Prod.*, 60: 925-8

Nagle D.G. and Paul V.J. (1998) Chemical defence of a marine cyanobacterial bloom. *J. Exp. Mar. Biol. Ecol.*, 225: 29-38

Nagle D.G. and Paul V.J. (1999) Production of secondary metabolites by filamentous tropical marine cyanobacteria: Ecological functions of the compounds. *J. Phycol.*, 35: 1412-1421

Nagle D.G., Camacho F.T. and Paul V.J. (1998) Dietary preferences of the opisthobranch mollusc *Stylocheilus longicauda* for secondary metabolites produced by the tropical cyanobacterium *Lyngbya majuscula*. *Mar. Biol.*, 132: 267-273

Nagle D.G., Park P.U. and Paul V.J. (1997) Pitiamide A, a new chlorinated lipid from a mixed marine cyanobacterial assemblage. *Tetrahedron Lett.*, 38: 6969-6972

Nagle D.G., Paul V.J. and Roberts M.A. (1996) Ypaoamide, a new broadly acting feeding deterrent from the marine cyanobacterium *Lyngbya majuscula*. *Tetrahedron Lett.*, 37: 6263-6266

Nagle D.G., Zhou Y.-D., Park P., Paul V., Rajbhandari I., Duncan C. and Pasco D. (2000) A new indanone from the marine cyanobacterium *Lyngbya majuscula* that inhibits hypoxia-induced activation of the VEGF promoter in Hep3B cells. *J. Nat. Prod.*, 63: 1431-1433

Nakayasu M., Fujiki H., Mori M., Sugimura T. and Moore R.E. (1981) Teleocidin, lyngbyatoxin A and their hydrogenated derivatives, possible tumor promoters, induce terminal differentiation in HL-60 cells. *Cancer Lett.*, 12: 271-7

Negri A. and Jones G. (1995) Bioaccumulation of paralytic shellfish poisoning (PSP) toxins from the cyanobacterium *Anabaena circinalis* by the freshwater mussel *Alathyria condola*. Toxicon 33: 667-678

Nelissen B., Wilmotte A., Neefs J.M. and DeWachter R. (1994) Phylogenetic relationships among filamentous helical cyanobacteria investigated on the basis of 16S ribosomal RNA gene sequence analysis. *Syst. Appl. Microbiol.*, 17: 206-210

National Health and Medical Research Council (2000) National Statement on Ethical Conduct in Research Involving Humans. Commonwealth of Australia, Canberra.

Nilsson E., Mikaelsson B. and Andersson S. (1985) Atopy, occupation and domestic work as risk factors for hand eczema in hospital workers. *Contact Dermatitis*, 13: 216-223

Nixon R. (1996) Contact dermatitis and occupational skin disease. *Med. J. Aust.*, 165: 47-52

Nogle L. and Gerwick W.H. (2003) Diverse secondary metabolites from a Puerto Rico collection of *Lyngbya majuscula*. *J. Nat. Prod.*, 66: 217-220

Nogle L., Okino T. and Gerwick W.H. (2001a) Antillatoxin B, a neurotoxic lipopeptide from the marine cyanobacterium *Lyngbya majuscula*. *J. Nat. Prod.*, 64: 983-985

Nogle L., Williamson R. and Gerwick W.H. (2001b) Somamides A and B, two new dessipeptide analogues of dolastatin 13 from a Fijian cyanobacterial assemblage of *Lyngbya majuscula* and *Schizothrix* species. *J. Nat. Prod.*, 64: 716-719

Novo M., Hammarstrom A. and Janlert U. (1999) Does low willingness to respond introduce a bias? Results from a socio-epidemiological study among young men and women. *Int. J. Soc. Welfare*, 8: 155-163

Oberemm A., Fastner J. and Steinberg E. (1997) Effect of microcystin-LR cyanobacterial crude extracts on embryo-larval development of zebrafish (*Danio rerio*). *Water Res.*, 31: 2918-2921

Ohuchi K., Hirasawa N., Takahashi C., Watanabe M., Tsurufuji S., Fujiki H., Sugimura T. and Christensen S. (1986) Synergistic stimulation of histamine release from rat peritoneal mast cells by 12-O-tetradecanoylphorbol 13-acetate (TPA)-type and non-TPA-type tumor promoters. *Biochim. Biophys. Acta*, 887: 94-99

Ohuchi K., Watanabe M., Takahashi C., Hayashi Y., Hirasawa N., Tsurufuji S., Fujiki H. and Sugimura T. (1987) Analysis of tumor-promoter-induced inflammation in rats: Participation of histamine and prostaglandin E-2. *Biochim. Biophys. Acta*, 925: 156-163

Oliver J., Warner R. and Cleland D. (1983) Distribution of *Vibrio vulnificus* and other lactose-fermenting vibrios in the marine environment. *Appl. Environ. Microbiol.*, 45: 985-998

O'Neil J., Albert S., Ahern K., Ahern C., Hey K., Lukondeh T., Moody P., Osborne N., Pointon S., Powell B., Rose A., Salmon T., Shaw G., Watkinson A., Waite D. and Dennison W. (2002) *Lyngbya majuscula* blooms in coastal Australian waters: potential upstream causes and downstream effects. 10th International Conference on Harmful Algae St. Pete Beach, Florida, USA pp. addendem

O'Neil J.M. (1998) The colonial cyanobacterium *Trichodesmium* as a physical and nutritional substrate for the harpacticoid copepod *Macrosetella gracilis*. *J. Plankton Res.*, 20: 43-59

O'Neil, J.M., Shaw, G.R. & Dennison, W. (2000) Blooms of the Toxic Cyanobacteria *Lyngbya majuscula* in Coastal Queensland Waters. In *9th International Conference on Harmful Algal Blooms*. pp. 43. Hobart, Tasmania.

Onodera H., Satake M., Oshima Y., Yasumoto T. and Carmichael Wayne W. (1997) New saxitoxin analogues from the freshwater filamentous cyanobacterium *Lyngbya wollei*. *Nat. Toxins*, 5: 146-151

Orjala J. and Gerwick W.H. (1996) Barbamide, a chlorinated metabolite with molluscicidal activity from the Caribbean cyanobacterium *Lyngbya majuscula*. *J. Nat. Prod.*, 59: 427-30

Orjala J. and Gerwick W.H. (1997) Two quinoline alkaloids from the Caribbean cyanobacterium *Lyngbya majuscula*. *Phytochemistry*, 45: 1087-1090

Orjala J., Nagle D. and Gerwick William H. (1995a) Malyngamide H, an ichthyotoxic amide possessing a new carbon skeleton from the Caribbean cyanobacterium *Lyngbya majuscula*. *J. Nat. Prod.*, 58: 764-768

Orjala J., Nagle D.G., Hsu G. and Gerwick W., H. (1995b) Antillatoxin: An exceptionally ichthyotoxic cyclic lipopeptide from the tropical cyanobacterium *Lyngbya majuscula*. *J. Am. Chem. Soc.*, 117: 8281-8282

Orr P. and Jones G. (1998) Relationship between microcystin production and cell division rates in nitrogen-limited *Microcystis aeruginosa* cultures. *Limnol. Oceanog.*, 43: 1604-1614

Orsini M., Pannell L. and Erikson K. (2001) Polychlorinated acstamides from the cyanobacterium *Microcoeleus lyngbyaceus*. *J. Nat. Prod.*, 64: 572-577

Osborne N.J.T. and McNeill D.M. (2001a) Characterisation of *Leucaena* condensed tannins by size and protein precipitation capacity. *J. Sci. Food Agric.*, 81: 1113-1119

Osborne, N.J.T., Webb, P.M. and Shaw, G.R. (2001b). The toxins of *Lyngbya majuscula* and their human and ecological health effects. *Environ. Int.* 27: 381-392.

Osborne N.J.T., Webb P.M. and Shaw G.R. (2001c) Environmental Toxicology of the Cyanobacterium *Lyngbya majuscula*. 5th International Conference on Cyanobacterial Toxins Noosa, Australia

Osborne N.J., Webb P.M., Moore M.R. and Shaw G.R. (2001d) Environmental Toxicology of the Cyanobacterium *Lyngbya majuscula*. *Toxicology*, 164: 203

Papanaoum K., Marshmann G., Gordon L.A., Lumb R. and Gordon D.L. (1998) Concurrent infection due to *Shewanella putrefaciens* and *Mycobacterium marinum* acquired at the beach. *Aust. J. Dermatol.*, 39: 92-5

Patrick E., Maibach H. and Burkhalter A. (1985) Mechanism of chemically induced skin irritation. *Toxicol. Appl. Pharmacol.*, 81: 476-490

Paul V.J. (2001) Final Report: Chemical Ecology of Cyanobacteria on the Tropical Reefs in Guam. US Environmental Protection Agency, Washington.

Pearlman E., Garhart C., Grand D., Diaconu E., Strine E. and Hall L. (1999) Temporal recruitment of neutrophils and eosinophils to the skin in a murine model for onchocercal dermatitis. *Am. J. Trop. Med. Hyg.*, 61: 14-18

Pennings S.C., Weiss A.M. and Paul V.J. (1996) Secondary metabolites of the cyanobacterium *Microcoleus lyngbyaceus* and the sea hare *Stylocheilus longicauda*: Palatability and toxicity. *Mar. Biol.*, 126: 735-743

Pflugmacher S., Best J., Wiegand C. and Codd G.A. (2000) Inhibition of human recombinant glutathione S-transferase activity by cyanobacterial lipopolysaccharides - Supporting the influence of lipopolysaccharides on the toxicity of microcystin-LR. 9th International Conference on Harmful Algal Blooms Hobart, Australia pp. 200

Philipp R. (1991) Risk assessment of exposure to cyanobacteria. *Environ. Health*, 99: 80-83

Philipp R. (1992) Health risks associated with recreational exposure to blue-green algae (cyanobacteria) when dinghy sailing. *Health and Hygiene*, 13: 110-114

Philipp R., Brown M. and Francis F. (1992) Health risks associated with recreational exposure to blue-green algae (cyanobacteria) when windsurfing and fishing. *Health and Hygiene*, 13: 115-119

Philipp R., Evens E., Hughes A., Grisdale S., Enticott R. and Jephcott A. (1985) Health risks of snorkel swimming in untreated water. *Int. J. Epidemiol.*, 14: 624-627

Pierce R., Henry M., Blum P., Lyons J., Cheng Y., Yazzie D. and Zhou Y. (2003) Brevetoxin concentrations in marine aerosol: human exposure levels during a Karenia brevis harmful algal bloom. *Bull. Environ. Contam. Toxicol.*, 70: 161-165

Pietsch C., Wiegand C., Ame M., Nicklisch A., Wunderlin D. and Pflugmacher S. (2001) The effects of a cyanobacterial crude extracts on different aquatic organisms: Evidence for cyanobacterial toxin modulating factors. *Environ. Toxicol.*, 16: 535-542

Pilotto L.S., Douglas R.M., Burch M.D., Cameron S., Beers M., Rouch G.J., Robinson P., Kirk M., Cowie C.T., Hardiman S., Moore C. and Attwell R.G. (1997) Health effects of exposure to cyanobacteria (blue-green algae) during recreational water-related activities. *Aust. N. Z. J. Public Health*, 21: 562-566

Pilotto L.S., Kliewer E.V., Davies R.D., Burch M.D. and Attewell R.G. (1999) Cyanobacterial (blue-green algae) contamination in drinking water and perinatal outcomes. *Aust. N. Z. J. Public Health*, 23: 154-8

Pitois S., Jackson M. and Wood B. (2000) Problems associated with the presences of cyanobacteria in recreational and drinking waters. *Int. J. Environ. Health Res.*, 10: 203-218

Praud A., Valls R., Piovetti L. and Banaigs B. (1993) Malyngamide G: Proposition de structure pour un nouvel amide chlore d'une algue bleu-verte epiphyt de *Cystoseira crinita*. *Tetrahedron Lett.*, 34: 5437-5440

Princep M., Thompson R., West M. and Wylie B. (1996) Tolypodiol, and antiinflammatory diterpenoid from the cyanobacterium *Tolypothrix nodosa*. *J. Nat. Prod.*, 59: 786-788

Prinsep M., Caplan F., Moore R.E., Patterson G., Honkanen R. and Boynton A. (1992) Microcystin-LA from a blue-green-alga belonging to the Stigonematales. *Phytochemistry*, 31: 1247-1248

Queensland Government (1994) Environmental Protection Act, Parliament of Queensland, Brisbane

Queensland Government (1997) Environmental Protection (Water) Policy, Parliament of Queensland, Brisbane

Queensland Parks Service (2001). First aid records, Fraser Island. pp. 176: Queensland Parks and Wildlife Service, Queensland Government.

Quackenbush S., Work T., Balazs G., Casey R., Rovnal J., Chaves A., duToit L., Baines J., Parrish C., Bowser P. and Casey J. (1998) Three closely related herpes viruses are associated with fibropapillomatoosis in marine turtles. *Virology*, 246: 392-399

Ramos O.F., Masucci M.G. and Klein E. (1984) Activation of cytotoxic activity of human blood lymphocytes by tumor-promoting compounds. *Cancer Res.*, 44: 1857-1862

Ramsing D. and Agner T. (1997) Effect of water on experimentally irritated human skin. *Br. J. Dermatol.*, 136: 364-367

Rapala J., Lahti K., Sivonen K. and Niemelä S. (1993a) Biodegradation and adsorption on lake sediments of cyanobacterial hepatotoxins and anatoxin-a. *Lett. Appl. Microbiol.*, 19: 423-428

Rapala J., Sivonen K. and Niemelä S. (1995) Comparison of toxin production by hepatotoxic and neurotoxic *Anabaena* spp. 1st International Congress on Toxic Cyanobacteria (Blue-Green Algae) Ronne, Denmark pp. 54

Rapala J., Sivonen K., Lyra C. and Niemelä S. (1993b) Anatoxin-a concentration in *Anabena* and *Aphanizomenon* at different environmental conditions and comparison of growth by toxic and non-toxic *Anabena* strains, a laboratory study. *J. Appl. Phycol.*, 5: 581-591

Rapala J., Sivonen K., Lyra C. and Niemelä S. (1997) Variation of microcystins, cyanobacterial hepatotoxin, in *Anabena* spp. as a function of growth stimuli. *Appl. Environ. Microbiol.*, 63:

Reed J., Ghandially R. and Elias P. (1995) Skin type, but neither race nor gender, influence epidermal permeability barrier function. *Arch. Dermatol.*, 131: 1134-1138

Ressom R., Soong F.S., Fitzgerald J., Turczynowicz L., el Saadi O., Roder D., Maynard T. and Falconer I. (1994) *Health Effects of Toxic Cyanobacteria (Blue-Green Algae)*. Commonwealth of Australia, Canberra.

Reynolds C.S. (1984) Plankton periodicity: the interactions of form, function and environmental variability. *Freshwater Biol.*, 14: 111-142

Rippka R., Deruelles J., Waterbury J., Herdman M. and Tanier R. (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol.*, 111: 1-61

Robarts R.D. and Zohary T. (1987) Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. *N.Z. J. Marine Freshwater Res.*, 21: 391-399

Robinson C.P., Franz D.R. and Bondura M.E. (1991) Effects of lyngbyatoxin A from the blue-green alga Lyngbya majuscula on rabbit aorta contractions. *Toxicon*, 29: 1009-1018

Robinson M. (1999) Population differences in skin structure and physiology and the susceptibility to irritant and allergenic contact dermatitis: implications for skin safety testing risk assessment. *Contact Dermatitis*, 42: 134-143

Robinson M. (2001) Intra-individual variations in acute and cumulative skin irritation responses. *Contact Dermatitis*, 45: 75-83

Robinson M. (2002) Population differences in acute skin irritation responses. *Contact Dermatitis*, 46: 86-93

Roe F. (1957) The binding of [1-^{14}C] phenol to the proteins of mouse skin. *Br. Empire Cancer Campaign Res.*, 35: 507-512

Rohold A.E., Halkier Sorensen L., Andersen K.E. and Thestrup Pedersen K. (1994) Nickel patch test reactivity and the menstrual cycle. *Acta-Derm-Venereol*, 74: 383-5

Romans, K.M., Carpenter, E.J. & Bergman, B. (1994). Buoyancy regulation in the colonial diazotrophic cyanobacterium *Trichodesmium tenue*: Ultrastructure and storage of carbohydrate, polyphosphate, and nitrogen. *J. Phycology*, 30, 935-942.

Romay C., Armesto J., Remirez D., Gonzalez R., Ledon N. and Garcia I. (1998a) Antioxidant and anti-inflammatory properties of C-phycocyanin from blue-green algae. *Inflamm. Res.*, 47: 36-41

Romay C., Ledon N. and Gonzalez R. (1998b) Further studies on anti-inflammatory activity of phycocyanin in some animal models of inflammation. *Inflamm. Res.*, 47: 334-338

Roskov K., Maibach H. and Guy R. (1989) The effect of aging on percutaneous absorption in man. *J. Pharmacokinet. Biopharm.*, 17: 617-630

Rossi J., V., Roberts Mary A., Yoo Hye D. and Gerwick William H. (1997) Pilot scale culture of the marine cyanobacterium *Lyngbya majuscula* for its pharmaceutically-useful natural metabolite curacin A. *J. Appl. Phycol.*, 9: 195-204

Rystedt I. (1985) Hand eczema in patients with history of atopic manifestations in childhood. *Acta Derm. Venereol.*, 65: 305-312

Saadoun I., Schrader K. and Blevins W.T. (2001) Environmental and nutritional factors affecting geosmin synthesis by *Anabaena* sp. *Water Res.*, 35: 1209-1218

Sadek S., Ittawa I. and Martello R. (1986) Culture of mullet species in ponds receiving iron crush effluents at El-Baharia Oasis, Egypt. *Aquaculture*, 59: 23-30

Sakamoto H., Terada M. and Fujiki H. (1981) Stimulation of prostaglandin production and choline turnover in HeLa cells by lyngbyatoxin A and dihydroteleocidin B. *Biochem. Biophys. Res. Commun.*, 102: 100-107

Saleem M., Ahmed S.-U., Alam A. and Sultana S. (2001) *Tephrosia purpurea* alleviates phorbol ester-induced tumor promotion response in murine skin. *Pharmacol. Res.*, 43: 135-144

Salmon T.P., Waite T.D. & Nielan B.A. (2002) Phylogenetic analysis of *L. majuscula* bloom ("Mermaid's Hair"). 5th International Conference on Cyanobacterial Toxins, Noosa, Australia

Sams W.M. (1949) Seabather's eruption. *Arch. Dermatol.*, 60: 227-237

Sato S., Paranagua M. and Eskinazi E. (1966) On the mechanism of red tide of *Trichodesmium* in Recife northeastern Brazil, with some considerations of the relation to the human disease, "Tamandare fever". *Trabalhos do Instituto Oceanographico da Universidade do Recife*, 5/6: 7-49

Schindler D.W. (1977) Evolution of phosphorus limitation in lakes. Science 195:260-262

Scholin C.A., Gulland F., Doucette G.J., Benson S., Busman M., Chavez F.P., Cordaro J., DeLong R., De Vogelaere A., Harvey J., Haulena M., Lefebvre K., Lipscomb T., Loscutoff S., Lowenstine L.J., Marin R., Miller P.E., McLellan W.A., Moeller P.D.R., Powell C.L., Rowles T., Silvagni P., Silver M., Spraker T., Trainer V. and Van Dolah F.M. (2000) Mortality of sea lions along the central California coast linked to a toxic diatom bloom. *Nature*, 403: 80-84

Scholtz J. (1965) Management of atopic dermatitis. *Californian Medicine*, 102: 210-216

Schwabe W., Weihe A., Henning M., Börner T. and Kohl J. (1988) Plasmids in toxic and nontoxic strains of the cyanobacterium *Microcystis aeruginosa*. *Curr. Microbiol*, 17: 133-137

Sellner K. (1997) Trophodynamics of marine cyanobacterial blooms. In: *Trichodesmium and other diazotrophs* (Eds. Carpenter E, Capone DG and Rueter JG). Kluwer Academic Publishers p. 357

Shen X., Lam P., Shaw G. Wickramasinghe W. (2002) Genotoxicity investigation of a cyanobacterial toxin, cylindrospermopsin. *Toxicon.* 40: 1499-1501

Smith V.H. (1983) Low nitrogen to phosphorus ratios favour dominance by blue-green algae in lake phytoplankton. *Science* 221:669-671.

South East Queensland Regional Water Quality Management Strategy (2001) Update of results from *Lyngbya* scientific tasks. South East Queensland Regional Water Quality Management Strategy, Brisbane.

Serdula M., Bartilini G., Moore R.E., Gooch J. and Wiebenga N. (1982) Seaweed itch on windward Oahu. *Hawaii Med. J.*, 41: 200-201

Shenefelt P. (1996) Epidemiology of irritant contact dermatitis. In: *The Irritant Contact Dermatitis Syndrome* (Eds. van der Walk P. and Maibach H.). CRC Press, Boca Raton, pp. 17-22

Shimomura K., Mullinix M.G., Kakunaga T., Fujiki H. and Sugimura T. (1983) Bromine residue at hydrophilic region influences biological activity of aplysiatoxin, a tumor promoter. *Science*, 222: 1242-4

Shmunes E. (1986) The role of atopy in occupational skin diseases. *Occupational Medicine State of Art Review*, 1: 219-228

Silva S., M. F. and Pienaar R.N. (1997) Epipelic marine cyanophytes of Bazaruto Island, Inhambane, Mozambique. *S. Afr. J. Bot.*, 63: 459-464

Sims J.K. and Zandee van Rilland R.D. (1981) Escharotic stomatitis caused by the "stinging seaweed" *Microcoleus lyngbyaceus* (formerly *Lyngbya majuscula*). Case report and literature review. *Hawaii Med. J.*, 40: 243-8

Sims J.K., Brock J.A., Fujioka R., Killion L., Nakagawa L. and Greco S. (1993) *Vibrio* in stinging seaweed: potential infection. *Hawaii Med. J.*, 52: 274-5

Singh I.P., Milligan K.E. and Gerwick W.H. (1999) Tanikolide, a toxic and antifungal lactone from the marine cyanobacterium *Lyngbya majuscula. J. Nat. Prod.*, 62: 1333-1335

Sitachitta N. and Gerwick W.H. (1998) Grenadadiene and grenadamide, cyclopropyl-containing fatty acid metabolites from the marine cyanobacterium *Lyngbya majuscula. J. Nat. Prod.*, 61: 681-4

Sitachitta N., Williamson R. and Gerwick W. (2000) Yacunamides A and B, two new depsipeptides from an assemblage of the marine cyanobacteria *Lyngbya majuscula* and Schizothrix species. *J. Nat. Prod.*, 63: 197-200

Sivonen K. (1990) Effects of light, temperature, nitrate, orthophosphate, and bacteria on growth of and hepatatoxin production by *Oscillatoria agarhii* strains. *Appl. Environ. Microbiol.*, 56: 2658-2666

Sivonen K. (1996) Cyanobacterial toxins and toxin production. *Phycologia*, 35 (6 supplement): 12-24

Sivonen K. and Jones G. (1999) Cyanobacterial toxins. In: *Toxic Cyanobacteria in Water* (Eds. Chorus I and Bartram J). Spon, London, pp. 41-111

Sivonen K., Kononen K., Carmichael W., Dahlem A., Rinehart K., Kiviranta J. and Niememla S. (1989) Occurence of hepatotoxic cyanobacterium *Nodularum spumigena* in the Baltic Sea and the structure of the toxin. *Appl. Environ. Microbiol.*, 55: 1990-1995

Skulberg O., Carmichael W., Andersen R., Matsunaga S., Moore R.E. and Skulberg R. (1992) Investigations of a neurotoxic oscillatorialean strain (Cyanophycae) and its toxin. Isolation and characterisation of homoanatoxin-a. *Environ. Toxicol. Chem.*, 11: 321-329

Skulberg O.M., Carmichael W.W., Codd G.A. and Skulberg R. (1981) Taxonomy of toxic Cyanophyceae (Cyanobacteria). In: *The Water Environment, Algal Toxins Health* (Ed. Carmichael WW). Plenum Press, New York, pp. 145-164

Solomon A.E. and Stoughton R.B. (1978) Dermatitis from purified sea algae toxin (debromoaplysiatoxin). *Arch. Dermatol.*, 114: 1333-5

Soong F.S., Maynard E., Kirke K. and Luke C. (1992) Illness associated with blue-green algae. *Med. J. Aust.*, 156: 67

SPSS (2002) SPSS for Windows. 11.5.0 Chicago, USA

Stafford R.G., Mehta M. and Kemppainen B.W. (1992) Comparison of the partition coefficient and skin penetration of a marine algal toxin (lyngbyatoxin A). *Food Chem. Toxicol.*, 30: 795-801

Stanier R. and Cohen-Bazire G. (1977) Phototropic procaryotes: the cyanobacteria. In: *Annu. Rev. Microbiol.* (Eds. Starr M, Ingraham J and Balows A). Annual Reviews Inc., Palo Alto, pp. 225-274

Steele R. and Wilhelm D. (1966) The inflammatory reaction in chemical injury. I. Increased vascular permeability and erythema induced by various chemicals. *Br. J. Exp. Pathol.*, 47: 612-623

Steele R. and Wilhelm D. (1970) The inflammatory reaction in chemical injury. III. Leucocytosis and other histologic changes induced by superficial injury. *Br. J. Exp. Pathol.*, 51: 265-279

Steinman H. (1987) Water-induced prutitis. *Clin. Dermatol.*, 5: 41-48

Stewart I., Webb P., Schulter P., Shaw G. and Moore M. (2001) The epidemiology of recreational exposure to freshwater cyanobacteria - an observational study of recreational water users in Queensland and New South Wales. *Toxicology*, 164: 174

Strauss M. and Dierker R. (1987) Otitis Externa associated with aquatic activities (swimmer's ear). *Clin. Dermatol.*, 5: 103-111

Suber S., Wilhelm K. and Maibach H. (1991) In-vitro skin pharmacokinetics of acitretin: percutaneous absorption studies in intact and modified skin from three different species using different receptor solutions. *J. Pharm. Pharmacol.*, 43: 836-840

Suganuma M., Fujiki H., Tahira T., Cheuk C., Moore R.E. and Sugimura T. (1984) Estimation of tumor promoting activity and structure-function relationships of aplysiatoxins. *Carcinogenesis*, 5: 315-318

Suganuma M., Yatsunami J., Yoshizawa S., Okabe S. and Fujiki H. (1993) Absence of synergistic effects on tumor promotion in CD-1 mouse skin by simultaneous applications of two different types of tumor promoters, okadaic acid and teleocidin. *Cancer Res.*, 53: 1012-6

Sugimura T. (1986) Studies on environmental chemical carcinogenesis in Japan. *Science*, 233: 312-8

Sundararaman M., Averal H.I., Akbarsha M.A. and Subramanian G. (1994) Bio-activity of marine cyanobacteria in the animal-based systems: Modulation of food intake, body weight and some haematological characters. *Ann. Appl. Biol.*, 125: 195-206

Sunmap (1998). Fraser Island. Brisbane: The State of Queensland (Department of Natural Resources).

Tagami H. (1971/1972) Functional characteristics of aged skin . 1. Percutaneous absorption. *Acta Dermatol. Kyoto. Engl. Ed.*, 66/67: 19-21

Takashima M., Sakai H. and Arima K. (1962) A new toxic substance, teleocidin produced by *Streptomyces*. Part IV. Degradative studies of hydroteleocidin B and teleocidic anhydride. *Agric. Biol. Chem.*, 26: 669-678

Takenaka S. (2001) Covalent glutathione conjugation to cyanobacterial hepatotoxin microcystin-LR by F344 rat cytostolic and microsomal glutathione S-transferase. *Environ. Toxicol. Pharmacol.*, 9: 135-139

Tan L.T., Okino T. and Gerwick W.H. (2000) Hermitamides A and B, toxic malyngamide-type natural products from the marine cyanobacterium *Lyngbya majuscula*. *J. Nat. Prod.*, 63: 952-955

Tarczynska T., Nalecz-Jawecki G., Ramanowska-Duda Z., Sawicki J., Beattie K., Codd G.A. and Zalewski M. (2001) Tests for the toxicity assessment of cyanobacterial bloom samples. *Environ. Toxicol.*, 16: 383-390

Terao K., Ohmori S., Igarashi K., Ohtani I., Watanabe M., Harada K.-I., Ito E. and Watanabe M. (1994) Electron microscopic studies on experimental poisoning in mice induced by cylindrospermopsin isolated from blue-green alga *Umezakia natans*. *Toxicon*, 32: 833-843

Thacker R. and Paul V. (2001) Are benthic cyanobacteria indicators of nutrient enrichment? Relationships between cyanobacterial abundance and environmental factors on the reef flats of Guam. *Bull. Marine Sci.*, 69: 497-508

Thacker R., W., Nagle Dale G. and Paul Valerie J. (1997) Effects of repeated exposures to marine cyanobacterial secondary metabolites on feeding by juvenile rabbitfish and parrotfish. *Marine Ecol. Prog. Series*, 147: 21-29

Thompson J. and Khalil R. (2003) Gender differences in the regulation of vascular tone. *Clin Exp Pharmacol Physiol.*, 30: 1-15

Todd J.S. and Gerwick W.H. (1995b) Isolation of a cyclic carbonate, a gamma-butyrolactone, and a new indole derivative from the marine cyanobacterium *Lyngbya majuscula*. *J. Nat. Prod.*, 58: 586-589

Todd J.S., and Gerwick W.H. (1995a) Malyngamide I from the tropical marine cyanobacterium *Lyngbya majuscula* and the probable structure revision of stylocheilamide. *Tetrahedron Lett.*, 36: 7837-7840

Torokne A., Palovics A. and Bankine M. (2001) Allergenic (sensitization, skin and eye irritation) effects of freshwater cyanobacteria – experimental evidence. *Environ. Tox.* 16: 512-516

Treneva I., Asparuhova D., Dzhambazov B., Mladenov R. and Schirmer K. (2003) The freshwater cyanobacteria *Lyngbya aeruginosa-coerulea* produces compounds toxic to mice and to mammalian and fish cells. *Environ. Tox.*, 18: 9-20

Turner (1990) Pneumonia associated with contact with cyanobacteria. *Br. Med. J.*, 300: 1440-1441

Ueyama H., Sasaki I., Shimomura K. and Suganuma M. (1995) Specific protein interacting with a tumor promoter, debromoaplysiatoxin, in bovine serum is alpha-1-acid glycoprotein. *J. Cancer Res. Clin. Oncol.*, 121: 211-218

Utkilen H. and Gjolme N. (1995) Iron-stimulated toxin production in *Microcystis aeruginosa*. *Appl. Environ. Microbiol.*, 61: 797-800

van Asperen I., CM d.R., Schijven J., Oetomo S., Schelleken J., van Leeuwen N., Colle C., Havelaar A., Kromhout D. and Sprenger M. (1995) Risk of otitis externa after swimming in recreational fresh water lakes containing *Psuedomonas aeruginosa*. *Br. Med. J.*, 311: 1407-1410

van der Westhuizen A., Eloff J. and Kruger G. (1986) Effect of temperature and light (fluence rate) on the composition of the toxin of the cyanobacterium *Microcystis aeruginosa* (UV-006). *Arch. Hydrobiol.*, 108: 145-154

van der Westhuizen A.J. and Eloff J.N. (1983) Effect of culture age and pH of culture medium on the growth and toxicity of the blue-green algae *Microcystis aeruginosa*. *Zeitschrift fur Planzenphysiologie*, 110: 157-163

van Dolah F.M. (2000) Marine algal toxins: origins, health effects, and their increased occurrences. *Environ. Health Perspect.*, 108: 133-141

Vance B. (1977) Prophage induction in toxic *Microcystis aeruginosa* NRC-1. *J. Phycol. (Suppl.)*, 13: 70

von Schirnding Y.E., Kfir R., Cabelli V., Franklin L. and Joubert G. (1992) Morbidity among bathers exposed to polluted seawater. A prospective epidemiological study. *S. Afr. Med. J.*, 81: 543-6

Waldor M. and Mekalanos J. (1996) Lysogenic conversion by a filamentous phage encoding cholera toxin. *Science*, 272: 1910-1914

Ward C. and Codd G.A. (1999) Comparitive toxicology of four microcystins of different hydrophobicities to the protozoan, *Tetrahymena pyriformis*. *J. Appl. Microbiol.*, 86: 874-882

Waterman P. and Mole S. (1994) *Analysis of phenolic plant metabolites*. Blackwell Scientific, Oxford.

Walther, G.-R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T., Fromentin, J.-M., Hoegh-Guldberg, O. & Bairlein, F. (2002). Ecological responses to recent climate change. *Nature*, **416,** 389-395.

Watkinson A. (2000) *Ecophysiology of the marine cyanobacterium, Lyngbya majuscula (Oscilllatoriacae).* Honours Thesis, University of Queensland.

Webb G. (2001) Bacteriological assessment of beaches and river recreation areas in Redcliffe, Caboolture and the Sunshine Coast October 1999-April 2000. Februray 2001 37. Environmental Protection Agency, Queensland Government and Caboolture Shire Council, Brisbane.

Webb G. (2003) *Recreational Water Quality Monitoring at Popular Beaches, Lakes and Rivers in south-east Queensland.* May 2003 Environmental Technical Report no. 52. Waterways Scientific Services Branch, Queensland Environmental Protection Agency, Brisbane.

Wester R. and Maibach H. (1987) Animal species models for transdermal delivery. In: *Transdermal Delivery of Drugs* (Eds. Kydonieus A and Berner B). CRC Press, Inc., Boca Raton, pp. 61-70

Woodcock, A. (1948). Note concerning human respiratory irritation associated with high concentrations of plankton and mass mortality of marine organisms. *J. Marine Res.*, 7, 1-7

World Heath Organization (1990) Final report of the working group on health impact of human exposure to recreational marine waters. 27 February-2 March 1990 World Health Organisation, Rimini, Italy.

World Heath Organization (2000) Evaluation and use of epidemiological evidence for environmental health risk assessment: WHO guideline document. *Environ. Health Perspect.*, 108: 997-1002

World Heath Organization (2003) Dangerous aquatic organisms. In: Guidelines for safe recreational water environments Volume 1 – Coastal and fresh waters. WHO, Geneva pp. 173-188

Wilhelm D. and Maibach H. (1993) The effect of aging on the barrier function of human skin evaluated by *in vivo* transepidermal water loss measurements. In: *Noninvasive Methods for the Quantification of Skin Functions* (Eds. Frosch P. and Kligman A.). Springer, Heidelberg, pp. 181-189

Wilhelm K.-P. (1995) Irritant Dermatitis: Experimental Aspects. In: *Irritant Dermatitis. New Clinical and Experimental Aspects* (Eds. Elsner P. and Maibach H.). Karger, Basel, pp. 144-151

Wilhelm K.-P. and Maibach H.I. (1990) Factors predisposing to cutaneous irritation. *Dermatol. Clin.*, 8: 17-22

Williams (1987) Water quality in the Richmond River. No. 4. State Pollution Control Commission, Sydney, NSW, Australia.

Williamson R., Chapin E., Carr A., Gilbert J., Graupner P., Lewer P., McKamey P., Carney J. and Gerwick W. (2000) New Diffusion-Edited NMR experiments to expedite the dereplication of known compounds from natural product mixtures. *Organic Letters*, 2: 289-292

Wolfe G., Steinke M. and Kirst G. (1997) Grazing-activated chemical defence in a unicellular marine alga. *Nature*, 387: 894-897

Wong D.E., Terri L., Meinking B.A., Rosen L.B., Taplin D., Hogan D.J. and Burnett J. (1994) Seabather's eruption. *J. Am. Acad. Dermatol.*, 30: 399-406

Wood D. and Wood J. (1975) Pharmacologic and biochemical considerations of dimethyl sulfoxide. *Ann. N. Y. Acad. Sci.*, 243:

Woodcock A. (1948) Note concerning human respiratory irritation associated with high concentrations of plankton and mass mortality of marine organisms. *J. Mar. Res.*, 7: 1-7

Wright J. (2002) Attack and defend: The function and evolution of bioactive or toxic metabolites. 10th International Conference on Harmful Algal Blooms, St Pete's Beach, Florida, USA. pp. 1

Wu M., Milligan K.E. and Gerwick W.H. (1997) Three new malyngamides from the marine cyanobacterium *Lyngbya majuscula*. *Tetrahedron*, 53: 15983-15990

Wunderlich V., Sydow G. and Baumbach L. (1984) Enhancement of primate retrovirus synthesis by tumour promoters. *IARC Sci Publ*, 56: 299-304

Yamashita U. (1985) Effect of teleocidin on immune responses *in vitro*. *Jpn. J. Cancer Res.*, 76: 532-40

Yang H., Lee H. and Kim H. (1997) *Spirulina platensis* inhibits anaphylactic reaction. *Life Sci.*, 61: 1237-1244

Yasumoto T. (1998) Fish poisoning due to toxins of microalgal origins in the Pacific. *Toxicon*, 36: 1515-1518

Yoo H.D. and Gerwick W.H. (1995) Curacins B and C, new antimitotic natural products from the marine cyanobacterium *Lyngbya majuscula*. *J. Nat. Prod.*, 58: 1961-1965

Yoshida Y., Miyahara K. and Nakahara H. (1995) Relationships between the dominant phytoplankton ad DIN:DIP ratios in inland waters. *Fisheries Science Tokyo*, 61: 396-400

Yoshizawa Y., Tanojo H., Kim S. and Malibach H. (2001) Sea water or its components alter experimental irritant dermatitis in man. *Skin Res. Technol.*, 7: 36-39

Young J. and De Young L. (1989) Cutaneous models of inflammation for the evaluation of topical and systemic pharmacological agents. In: *Pharmacological Methods in the Control of Inflammation, Modern Methods in Pharmacology 5* (Eds. Chang J and Lewis A). Alan, R Liss, New York, NY, pp. 215-231

Zhang L., Hua, Longley Ross E. and Koehn Frank E. (1997) Antiproliferative and immunosuppressive properties of microcolin A, a marine-derived lipopeptide. *Life Sci.*, 60: 751-762

9. Appendix - Postal Survey

NRCET
National Research Centre for ENVIRONMENTAL TOXICOLOGY

Health of Ocean Users in Queensland

You are invited to participate in an
health study involving you and your ocean.

The aim of the project is to investigate the health of ocean users in Queensland to make a safer, healthier recreational environment for Queenslanders and their visitors.

If you agree to participate in this survey, you will be asked to fill out a short questionnaire on your health and the types of water activity you have undertaken over the last few months.

Your participation in this survey is voluntary. However we would like to encourage you to complete the survey and post it back to us, as the more people who agree to participate – the better our results will be.

- All information supplied will remain anonymous. Normal confidentiality reserved for medical records will be maintained.

- This study has been cleared by one of the human ethics committees of the University of Queensland in accordance with the National Health and Medical Research Council's guidelines. If you would like to speak to an officer of the University of Queensland not involved in the study, you may contact the Ethics Officer on 3365 3924. If you would like to discuss your participation in this study with project staff please call Mr Nick Osborne, 07 3274 9147 or Dr Glen Shaw, 07 3274 9120.

Thank you for your help

Questionnaire

Directions * please answer all the questions below
* indicate the best or closest answer with a tick or circle

General Information

1. Age _____years
2. Sex Male / Female
3. Occupation_____
4. Do you smoke **Y / N**
5. How many people live in your house? _____

Water Activities

6. Have you had contact with seawater (eg swimming, wading, fishing ...) from Moreton Bay / Pumicestone Passage in the last ***seven*** months. **Yes / No**

IF YES: Please go to **question 7**.
IF NO: Please move to **question 22** (last page)

7. If you have been in contact with the water what activities did you do?

walking on beach	☐	sailing	☐
swimming	☐	crabbing	☐
wading	☐	cast-netting	☐
snorkelling/diving	☐	fishing from jetty	☐
surfing	☐	fishing from shore	☐
water skiing	☐	fishing from boat	☐
jet skiing	☐	fishing in surf	☐
wind surfing	☐	other (please specify)_____	

8. Did you use:

	Never	sometimes	frequently
wetsuit	☐	☐	☐
sunsafe top	☐	☐	☐
sunscreen	☐	☐	☐

9. Did you suffer from any of the following ***after contact with the water***?

	no	mild	moderate	severe		no	mild	moderate	severe
skin itching	☐	☐	☐	☐	vomiting	☐	☐	☐	☐
redness	☐	☐	☐	☐	fever	☐	☐	☐	☐
burning	☐	☐	☐	☐	headache	☐	☐	☐	☐
swelling	☐	☐	☐	☐	mouth ulcers	☐	☐	☐	☐
wheals	☐	☐	☐	☐	sore eyes	☐	☐	☐	☐
blistering	☐	☐	☐	☐	discharge from eyes	☐	☐	☐	☐
abdominal pain	☐	☐	☐	☐	sore ears	☐	☐	☐	☐
nausea	☐	☐	☐	☐	discharge from ears	☐	☐	☐	☐
diarrhoea	☐	☐	☐	☐	other (please describe)				

If you answered 'no' to all of these please go to **question 22** otherwise please continue.
If you have had any symptoms after coming into contact with the water we would like to know more about what you were doing when you came into contact with the water.

10. What activities were you doing immediately before you had the symptoms?

walking on beach	☐	sailing	☐
swimming	☐	crabbing	☐
wading	☐	cast-netting	☐
snorkelling/diving	☐	fishing from jetty	☐
surfing	☐	fishing from shore	☐
water skiing	☐	fishing from boat	☐
jet skiing	☐	fishing in surf	☐
wind surfing	☐	other (please specify)_____	

11. Where were you when you came into contact with the water (eg Bongaree, Woorim, Pebble Beach, other? _____)

12. What time did you have contact with the water _____ am/pm

13. What time did you last have contact with the water _____ am/pm

14. About how long were you in the water altogether?

less than 30 min	☐
30-60 min	☐
over 60 min	☐

15. If you were in the water or on the beach, did you come into contact with any algae, weed or scum

Y / N Where on body?

hands	☐	face/head	☐
arms	☐	groin	☐
legs	☐	only under clothing	☐
torso	☐	other (please describe)_____	

16. Did you seek any medical advice for any of these symptoms?

	Yes	No		Yes	No
G. P.	☐	☐	hospital	☐	☐
pharmacist	☐	☐	specialist	☐	☐
			other (please specify)_____		

17. What was the cost of the treatment? $_____

18. Did you have any problems sleeping with these symptoms? **Y / N**

How long/ how many nights _____(how long)

19. Did you take any time off work? ☐ _____ (how long)

20. Would you return to the same location **Y / N**

21. Would you change your behaviour next time you go there **Y / N**

22. In the **last five years** have suffered from:

	Yes	No		Yes	No
eczema	☐	☐	asthma	☐	☐
dermatitis	☐	☐	allergies	☐	☐

23. Would you enter the water if there was weed/plant matter present **Y / N**
24. Have you experienced breathing or other problems whilst in the area of Bribie Island/Pumicstone Passage? **Y / N**

Were they any of these symptoms?

	never	once	sometimes	frequently
productive cough	☐	☐	☐	☐
dry cough	☐	☐	☐	☐
runny nose	☐	☐	☐	☐
sore throat	☐	☐	☐	☐
blocked sinuses	☐	☐	☐	☐
repeated sneezing	☐	☐	☐	☐
chest tightness	☐	☐	☐	☐
wheeze	☐	☐	☐	☐
sore eyes	☐	☐	☐	☐
discharge from eyes	☐	☐	☐	☐

other (please describe)_____

25. Have you heard of *Lyngbya*, fireweed or stinging blue-green algae **Y / N**

If yes, did you know it had the ability to cause

	Yes	No
skin rashes	☐	☐
eye irritations	☐	☐
diarrhoea	☐	☐
breathing problems	☐	☐
earache	☐	☐

26. Do you actively avoid entering the water in areas known to have *Lyngbya* / fireweed? **Y / N**