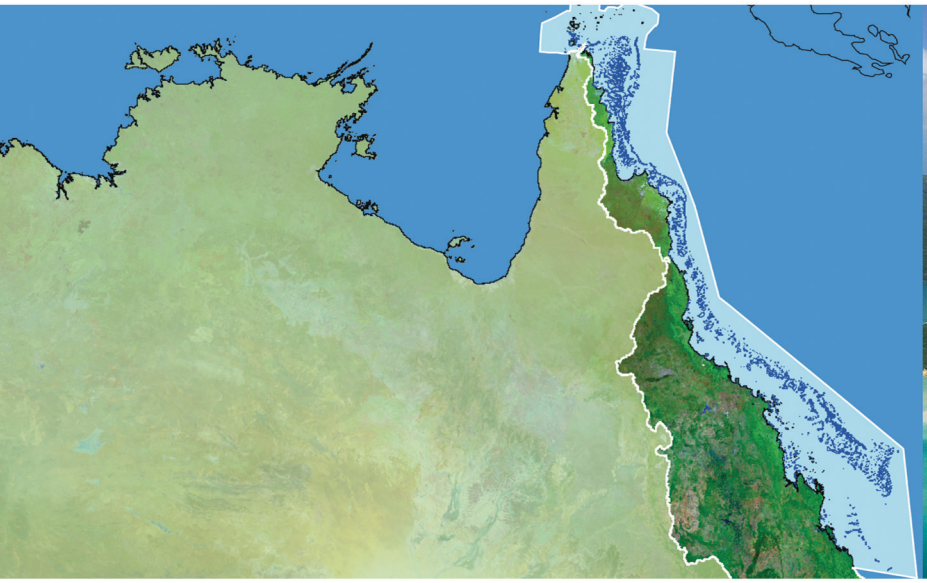
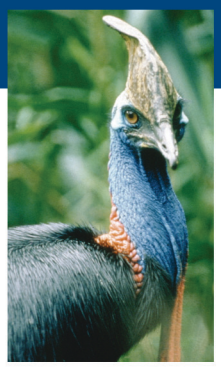
Commonwealth Environment Research Facilities

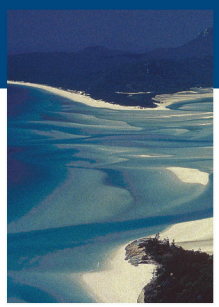
Marine and Tropical Sciences Research Facility



Assessment of the Threat of Toxic Microalgal Species to the Great Barrier Reef World Heritage Area

Compiled by Samantha Garrard, Kirsten Heimann and David Blair





JCU
JAMES COOK UNIVERSITY

Australian Government
Department of the Environment, Water, Heritage and the Arts

Assessment of the Threat of Toxic Microalgal Species to the Great Barrier Reef World Heritage Area

A Literature Review

Compiled by Samantha Garrard, Kirsten Heimann and David Blair

School of Marine and Tropical Biology,
James Cook University

Funded through the Australian Government's
Marine and Tropical Sciences Research Facility
Project 2.6.1 Identification and impact of
invasive species in the Great Barrier Reef

National Library of Australia Cataloguing-in-Publication entry:

Title:	Assessment of the threat of toxic microalgal species to the great barrier reef world heritage area : a literature review [electronic] / compiled by Samantha Garrard, Kirsten Heimann, David Blair.
Publisher:	Cairns, Qld. : Reef and Rainforest Research Centre, 2008.
ISBN:	9781921359033 (pdf)
Notes:	Bibliography.
Subjects:	Microalgae—Queensland—Great Barrier Reef. Coral reef biology—Queensland—Great Barrier Reef. Toxic marine algae—Queensland—Great Barrier Reef. Great Barrier Reef Region (Qld.)
Other Authors/Contributors:	Garrard, Samantha, 1978- Heimann, Kirsten, 1960- Blair, Tony, 1949-
Dewey Number:	579.809943

This report should be cited as:
Garrard, S., Heimann, K. and Blair, D. (2008) *Assessment of the Threat of Toxic Microalgal Species to the Great Barrier Reef World Heritage Area.* Literature Review. Marine and Tropical Sciences Research Facility Research Report Series. Reef and Rainforest Research Centre Limited, Cairns (28 pp.).

Published by the Reef and Rainforest Research Centre on behalf of the Australian Government's Marine and Tropical Sciences Research Facility.

The Marine and Tropical Sciences Research Facility (MTSRF) is part of the Australian Government's Commonwealth Environment Research Facilities programme. The MTSRF is represented in North Queensland by the Reef and Rainforest Research Centre Limited (RRRC). The aim of the MTSRF is to ensure the health of North Queensland's public environmental assets – particularly the Great Barrier Reef and its catchments, tropical rainforests including the Wet Tropics World Heritage Area, and the Torres Strait – through the generation and transfer of world class research and knowledge sharing.

This report is available for download from the
Reef and Rainforest Research Centre Limited website:
http://www.rrrc.org.au/publications/research_reports.html

Reef & Rainforest
RESEARCH CENTRE

Published in March 2008.

Contents

Overview

Prerequisites for the assessment of the threat of introduced and invasive marine pests to a particular ecosystem are knowledge of species present, their current distributions and abundances. This information, however, largely does not exist for microalgal communities in the Great Barrier Reef World Heritage Area (GBRWHA). The need for such baseline information was recognised by the Australian Ballast Water Management Advisory Council (ABWMAC), the Standing Committee on Agriculture and Resource Management (SCRAM), and the Australian and New Zealand Environment and Conservation Council (ANZECC). In recognition of these problems, baseline studies through port surveys have been conducted in Australia to define the state of introductions and occurrences of non-indigenous species. These efforts were coordinated largely by the CSIRO Centre for Research on Introduced Marine Species (CRIMP) and also involved several State agencies and research organisations.

Despite these surveys, knowledge on current microalgal community structure, occurrences and abundances of toxic microalgae in the GBRWHA remains scarce and incomplete, in particular in regard to introductions and invasiveness, which is defined in this review as range-expansions of existing microalgae due to habitat change. The main reasons for the paucity of information on toxic microalgae in the GBRWHA lies in the restriction of sampling efforts for only certain species, the sporadic and short term nature of port surveys, and the fact that samples were only analysed by traditional morphology-based techniques, which yield no information regarding toxicity of certain microalgal species. Taxonomical analysis based on morphology alone is also insufficient because the identification is often tentative at best, which is partially due to morphological plasticity within many microalgal species.

Due to the lack of developed molecular probes for fast and unambiguous identification of Great Barrier Reef (GBR)-specific microalgae, several issues and questions remain and may become more pressing under climate change scenarios. These are:

- Acknowledgement of the necessity for molecular tool development to overcome identification problems and expedite sample analyses specifically for the GBR.
- Acknowledgement of the need to develop tools to differentiate between toxic and non-toxic strains of identical morphology.
- What is the distribution of toxic strains?
- What are the economic and health threats for the region and how will climate change influence these risks?
- Are temperate regions more at risk from proliferation of introduced species of toxic microalgae than tropical regions, or is this perception merely a reflection of a greater human population density and associated eutrophication, as well as an imbalance of frequencies / durations of surveys between the regions?
- What is the current status of toxic microalgal invasions and realised modes of invasiveness in the GBRWHA?

This review will provide an overview of current knowledge of toxic microalgae with a focus on toxic dinoflagellates. It will briefly describe dinoflagellate characteristics, which impede identification and molecular tool development (see *Introduction*), provide an overview of microalgal toxins and associated human health issues, and summarise information available on the distribution of toxic microalgae in the GBRWHA and Australasia and their threats to the region. At the end, this review will also introduce suitable molecular techniques that need to be developed and utilised for GBR-specific toxic microalgal identifications in order to effectively predict the risks and threats that these organisms pose to the GBRWHA and the local human population now and under future temperature regimes.

Introduction

Microalgae are of considerable importance within marine ecosystems; they are significant contributors to primary productivity and food webs, some are endosymbiotic with, for example, scleractinian corals, anemones, foraminiferans, etc. Some are parasitic, and others can produce harmful toxins which can accumulate in shellfish and finfish and, upon consumption, cause human poisonings. Phytoplankton (microalgae living freely suspended in the water column) is estimated to fix 70% of the 2.2 x 10^5 tonnes of carbon fixed daily within the GBR (Furnas & Mitchell 1996), showing the importance of microalgae as a carbon sink.

In the past few years, research has focussed on the detection of toxic microalgae because of their negative impact on economies, costing fisheries millions of dollars per annum. The most prominent toxic microalgae are those that form harmful algal blooms (HABs). Blooms are defined as temporary and localised excessive proliferations of particular microalgal species. These blooms are designated to be harmful if they harm marine life and/or humans either through the production of toxins or by other means, e.g. clogging of fish gills. Sometimes blooms are also referred to as red or brown tides, because densities of organisms present discolour the water. The terms red or brown tides, however, are not synonymous with the term HABs because they may not have adverse effects on humans or marine life. It must be emphasised that the occurrence of HABs is not a prerequisite for adverse impacts of algal toxins on human health as toxins can be bioaccumulated in shellfish and reef fish and passed onto humans via the consumption of contaminated marine organisms. Toxin production and levels of toxicity is another research area having received much attention recently. However, the nature of toxin production, e.g. whether toxins are produced by microalgae themselves or by associated intra- or extracellular bacteria, or are modified upon ingestion, has still not been resolved. For the purpose of this review, we will assume toxins are produced directly by microalgae, dinoflagellate species or strains, because these have always been observed to be present in outbreaks.

In the past few decades, incidences of toxic microalgal outbreaks have increased and the greater geographical range of many species has been documented (Hallegraeff 2003). The genera *Gymnodinium* and *Alexandrium* receive the most research attention worldwide as they can form large blooms of more than twenty million cells per litre (Hackett *et al.* 2005) and are predominantly consumed by filter-feeding molluscs such as mussels and oysters. The toxins are lipophilic, bioaccumulate in fatty tissues of animals and are passed up the food chain where they can cause serious neurological symptoms and even death to higher order predators such as cetaceans (Doucette *et al*. 2006) and humans (Hallegraeff 1998).

Dinoflagellate lifecycles and modes of nutrition can be exceedingly diverse. Although they are classified as ‘algae’, approximately half the extant species do not contain chloroplasts and are thought to be obligate heterotrophs (Gaines & Elbrächter 1987). Of those remaining, the majority are mixotrophic, requiring organic compounds in addition to photosynthetic products to sustain growth. Phagotrophy is common in dinoflagellates, and many prey on both prokaryotic and eukaryotic prey.

Dinoflagellates are primarily haploid with vegetative reproduction dominating their lifecycle (Pfeister & Anderson 1987). Typically, during sexual reproduction gametes fuse to form diploid planozygotes, which enlarge and upon loss of flagella transform into hypnocysts, which sink to the sediments. Hypnocysts undergo a requisite period of dormancy before being able to hatch, although they may remain viable in the sediment for five to ten years (Anderson *et al*. 1995). Under the correct environmental conditions hypnocysts can germinate, entering the vegetative reproduction cycle. Excessive numbers of cell divisions are thought to occur under suitable climatic and nutrient conditions leading to the initiation of

a bloom. Hypnocysts have been proposed to be 'seeds' for initiating blooms (Hallegraeff *et al*. 1998). Some species also produce vegetative temporary cysts by shedding their flagella and rounding of the cell (Marasovic 1989). Vegetative cyst formation often occurs when environmental conditions deviate greatly from the optimum, in the absence of another mating compatible strain, and is thought to function as a protective mechanism (Marasovic 1989, Garces *et al*. 2002). Although these are the lifecycle stages of typical free living dinoflagellates, there are many variants on this. The heterotrophic dinoflagellate *Pfiesteria piscida*, nicknamed the 'cell from hell', has caused the death of billions of fish in estuarine waters within the USA (mode of action, e.g. toxin production, currently unknown and disputed) (Burkholder *et al.* 1992) and is thought to have the most complex life cycle with up to 24 lifecycle stages. The life cycle of *Pfiesteria* includes flagellated, and several amoeboid and cyst stages, which are all morphologically dissimilar to the vegetative cell (Burkholder *et al*. 1992). The complex life cycle of dinoflagellates and the occurrence of life cycle stages that often bear no resemblance to the vegetative cell generating them, as well as the occurrence of life cycle stages that are morphologically similar between different species, are major impediments to correct identification using light microscopy. Even under scanning electron microscopy, differences between cysts of different species can be imperceptible to the untrained eye and therefore experts are needed for correct identification of species present.

Dinoflagellates are unusual organisms in that they show a mixture of prokaryotic and eukaryotic features. They can contain more than a hundred chromosomes, which remain condensed throughout the cell cycle except for a short period in which they uncoil to allow replication (Dodge 1966). Their genome is extremely large and a haploid nucleus can contain over sixty times more DNA than a human diploid nucleus (Spector 1984). Unlike other eukaryotic cells they do not contain nucleosomes (Herzog & Soyer 1981), although histone-like proteins (HLPs) have been found within the nucleus (Rizzo 1981). Phylogenetic analysis of dinoflagellate HLPs show that they are closely related to proteobacterial HLPs and may have been acquired by lateral gene transfer (Hackett *et al*. 2005).

To date, efforts to identify genes that might code for toxins have been unsuccessful. The large genome size of dinoflagellates has hindered genome projects aimed to reveal the genes responsible for toxin production. Hackett *et al.* (2005) generated and analysed 10,885 expressed sequence tag (EST) sequences from *Alexandrium tamarense*, but no genes involved in toxin production were identified.

Table 1: ABWMAC Target Toxic Dinoflagellate Species (Hewitt & Martin 2001).

Species	Potential Origin
Alexandrium catenella	Global temperate
Alexandrium minutum	Mediterranean, Atlantic Europe
Alexandrium tamarense	Global temperate
Gymnodinium catenatum	East Pacific, Northern Europe

Bloom-forming toxic dinoflagellates are intensely researched due to their invasive nature, the history of recent introductions, huge economic losses and their risk to human health. The ABWMAC table of target marine pest species lists four toxic dinoflagellates (Table 1) thought to have been introduced to Australia through semi-dry and dry ballast and ballast water. Despite the temperate latitudes in which these organisms occur, they have been the focal point even in surveys of tropical Australian ports. A few of these temperate species have been used in development of molecular taxonomic tools. Although range expansions of these organisms is theoretically possible due to suitable habitat structure, e.g. in the Port of Townsville (CSIRO 1998), there is clearly a need to analyse toxic microalgal abundance and occurrence on the GBR by traditional means and with molecular techniques, to capture the

possible introduction of toxic tropical microalgae and potential range expansions of existing GBR-toxic microalgae (see next section) under changed climatic conditions to protect the health of the local community.

Toxic Microalgae Involved in Human Poisonings

There are five main human poisonings caused by consumption of seafood contaminated by microalgal toxins; four are mainly attributable to the presence of toxic dinoflagellates. These are ciguatera fish poisoning (CFP), paralytic shellfish poisoning (PSP) diarretic shellfish poisoning (DSP), and neurotoxic shellfish poisoning (NSP). Amnesiac shellfish poisoning (ASP) occurs through the consumption of seafood contaminated by toxic diatoms. The causative agents for other minor poisonings, e.g. azaspiriscid poisoning, are unknown (Daranas *et al*. 2001). We will therefore restrict this review to the five main poisonings (CFP, NSP, PSP, DSP and ASP), as they are likely to be the area of greatest concern within the GBR Ecosystem. Table 2 summarises microalgal species, their toxins, and current distributions relevant to Australia for the five main poisoning events. Those species considered of immediate or future concern to the GBR are indicated with an asterisk. Relevant references are cited in the text.

Although poisoning events are reportable, except for NSP, which is reportable only in Florida and only since 1999, attempts to quantify them are hindered by overlapping symptoms between the differing types of seafood poisoning and the fact that affected individuals often only show some of the symptoms, leading to misdiagnoses. All seafood poisonings, regardless of type, include symptoms such as nausea and gastrointestinal discomfort. Such symptoms can have many other unrelated causes and it is likely that the real number of cases is largely underestimated (e.g. weaker poisoning events are not diagnosed). Typically, misdiagnosis is only detected when numbers of patients with similar symptoms increase sharply in a short period of time. Authorities and the public are alerted more efficiently when regular monitoring programmes, aimed to quantify toxic microalgal abundances and the toxin content in shellfish beds, are in place within the region. While these procedures do little to reduce economic losses, at least human health is far better protected. It needs to be emphasised that there are no microalgal toxin monitoring programs in place for the GBRWHA, leaving the local population largely oblivious to potential danger from seafood poisoning and therefore unprotected.

Ciguatera Fish Poisoning (CFP)

Ciguatera is endemic to tropical and sub-tropical regions, and ciguateric fish are predominantly reef or oceanic fish species which feed on the reef. Ciguatera fish poisoning caused by the consumption of contaminated finfish is probably the most commonly reported seafood poisoning (Lehane & Lewis 2000) and exhibits a diverse array of symptoms. Some gastrointestinal symptoms can include diarrhea, nausea, vomiting and abdominal pains, whilst neurological symptoms can include paraesthesia, dysaesthesia, temperature reversal, pruritus and headaches and cardiovascular symptoms such as bradycardia and hypotension (Calvert 1991, Lehane & Lewis 2000). In some cases neurological effects may persist for months or years (Gillespie *et al.* 1986). It is suggested that fifty thousand or more cases occur globally per annum (Lehane & Lewis 2000, Daranas *et al.* 2001). The numbers of diagnosed ciguatera poisonings is, however, thought to be vastly underestimated as the symptoms vary greatly leading to frequent misdiagnosis (Lewis 2001). The low number of human fatalities from ciguatera poisoning may be attributable to fish mortalities at higher levels of ciguatera contamination (Lewis 1992).

Table 2: Summary of toxic microalgal species, their toxins and their distributions.

Ciguatera fish poisoning (CFP)		
Species	***Toxin***	***Distribution***
*Gambierdiscus toxicus**	• Gambiertoxins (precursor for ciguatera toxin? toxins are lipid soluble) • Maitotoxin (water soluble toxin)	Tropical, Tahiti, GBR
Ostreopsis spp.*	• Ostreotoxins (precursor to ciguatera toxins?) • Palytoxin-like analogues	Tropical, Indian Ocean, GBR (reported), Japan, New Zealand
oolia spp.*	• Cooliatoxins (precursors to ciguatera toxins?)	Tropical, GBR (likely), New Zealand
Neurotoxic shellfish poisoning (NSP)		
Species	***Toxin***	***Distribution***
Karenia brevis	• Brevetoxins • Hemolytic toxins (both toxins are lipid soluble, heat and acid stable)	Gulf of Mexica, Carribean, Texas, Louisiana, East coast of Florida, North and South Carolina
Paralytic shellfish poisoning (PSP)		
Species	***Toxin***	***Distribution***
*Alexandrium catenella**	• Saxitoxin and saxitoxin-analogues (water soluble toxin, of bacterial origin?)	Subarctic to tropical, Japan, New South Wales, Victoria, New Zealand
Alexandrium minutum		Mediterranean Sea, South East Asia, South Australia, New Zealand
*Alexandrium tamarense**		Subarctic to tropical
Gymnodinium catenatum		Tasmania, New Zealand, Singapore
Pyrodinium bahamense var. *compressum**		Tropical, Philippines, Indonesia, Malaysia, Papua New Guinea, Brunei, GBR(?)
Diarrhetic shellfish poisoning (DSP)		
Species	***Toxin***	***Distribution***
Dinophysis spp.*	• Okadaic acid and derivates (lipophilic toxin, digestion product of dinophysis toxins?)	Japan, Tropical, GBR
Prorocentrum spp.*	• Okadaic acid and derivates	Tropical, GBR
Amnesiac shellfish poisoning (ASP)		
Species	***Toxin***	***Distribution***
Pseudo-nitzschia spp.*	• Domoic acid (water soluble and heat stable toxin)	Temperate, Canada, Massachusetts, West coast of United States, Japan, New Zealand, Australia (?)

*Species considered of immediate or future concern for the GBRWHA.

The primary causative agent in ciguatera poisoning is *Gambierdiscus toxicus*, which produces gambiertoxins (GTXs) thought to be the precursors to ciguatoxins (CTXs) (Holmes *et al.* 1991). These toxins bioaccumulate in the food chain, with herbivorous fish and/or invertebrates acting as the vectors of ciguatoxins to carnivorous fish (Lewis 2001). CTXs are a family of lipid soluble, heat stable cyclic polyether molecules (Lewis 2001) and are structurally similar to brevetoxins (Holmes *et al*. 1991), although much more potent (Yasumoto & Murata 1993). It is thought that CTXs are oxidized to more potent forms upon ingestion (Lewis *et al*. 1991). As these toxins are lipid soluble, they primarily accumulate in the viscera of fish, although the majority of human poisonings result from fish muscle which is consumed more often and in higher quantities (Lehane & Lewis 2000). *Gambierdiscus toxicus* is also known to produce large quantities of maitotoxins, which are water-soluble. Although these are extremely toxic, they are not considered to contribute to ciguatera poisoning as they do not accumulate to a large degree in the muscle of fish (Lewis & Holmes 1993).

There has been some controversy on whether the diversity of symptoms may be wholly due to different types of ciguatoxins (Lewis 2001, Lewis 2006), or due to the presence of a combination of ciguatoxins and other dinoflagellate toxins present in fish (Yasumoto *et al.* 1987, Tindall *et al*. 1990, Morton *et al.* 1992). Legrand *et al*. (1990) found multiple toxins in the viscera of a moray eel, suggestive of the involvement of different genera and species. The toxic dinoflagellate genera *Ostreopsis* and *Coolia*, which coexist with *G. toxicus* (Steidinger 1993), are less well studied, but have also been implicated as causative agents in ciguatera poisoning (Yasumoto *et al*. 1987, Tindall *et al.* 1990, Morton *et al*. 1992, Daranas *et al.* 2001). Species from the genus *Ostreopsis* have been found to produce ostreotoxins and palytoxin analogues (Meunier *et al.* 1997, Lenoir *et al.* 2004), those from the genus *Coolia* produce cooliatoxins (Holmes *et al.* 1995). The genus *Prorocentrum*, which also coexists with *G. toxicus*, does not produce ciguatera toxins but okadaic acid derivatives (Morton *et al.* 1998). The okadaic acid group of toxins cause diarrhetic shellfish poisoning (Daranas *et al.* 2001) and, if consumed in conjunction with ciguatoxins, are believed to enhance ciguatera symptoms.

Dinoflagellates associated with ciguatera are distributed in the benthos often associated with macroalgae, coral rubble and fine silty sands on the reef (Lehane & Lewis 2000). Worldwide reports on the distribution of ciguatera are patchy. Some regions appear relatively free from ciguatera but are found next to regions with high incidence (Lewis 2001). This is also true for the distribution of ciguateric dinoflagellates. Gillespie *et al.* (1985) sampled *G. toxicus* on a number of reef sites along the Queensland coast (Figure 1) and found, although distributed across all sites tested, they were only present in large numbers at certain sites. Not all strains of *G. toxicus* are toxic; Tosteson *et al.* (1989) stated that 1 out of 5 clones was toxic, whilst Holmes *et al.* (1991) found 1 in 13. Very little is known about toxin production. It might be that certain strains are genetically programmed to be toxic whilst others are not. However, it is equally likely that toxin production could be controlled by environmental parameters (e.g. enhanced nutrient availability: eutrophic conditions). Toxin production may cease under laboratory culturing conditions and in some instances methods used for the detection of gambiertoxins may fail (Holmes *et al*. 1991).

The taxonomy of ciguatera-causing dinoflagellates is not well understood. Until recently it was thought that *Gambierdiscus toxicus* was the only species within the genus. However, currently, five different *Gambierdiscus* species are recognised (Chinain *et al*. 1999), which are all thought to produce ciguatoxins or their precursors. *Gambierdiscus toxicus* forms blooms at sea temperatures of ~30°C, whilst in the laboratory optimum growth rates were between 26-29 °C (Bomber *et al.* 1988). *Gambierdiscus toxicus* and *Ostreopsis lenticularis* bloomed in unison during a ciguatera outbreak in Tahiti (Bagnis *et al*. 1990). Reasons for bloom formation are not understood. While some authors argue that blooms of ciguateric dinoflagellates are not seasonal (linked to temperature) (Harbungs *et al.* 2001), others

believe that CFP will increase with increasing sea-surface temperatures (SSTs) (Hales *et al.* 1999, Chateau-Degat *et al.* 2005). Cateau-Degat *et al.* (2005) observed a correlation between increased *G. toxicus* abundance and higher sea surface temperatures. If the latter observation in response to increased temperature is correct, then the GBRWHA and its local community will be more affected by CFP then in the past, as predictions of the effect of climate change on reefs have forecast a phase shift to macroalgal dominance, one of the preferred habitats for CFP-associated dinoflagellates.

Neurotoxic Shellfish Poisoning (NSP)

NSP symptoms are similar to those of CFP and PSP. NSP is caused by brevetoxins found in the toxic dinoflagellate *Karenia brevis* (formerly *Gymnodinium breve*). NSP symptoms are gastroenteritis accompanied by neurological symptoms. *Karenia brevis* produces two types of lipid-soluble toxins, brevetoxins, which are responsible for the neurotoxic symptoms, and hemolytic toxins, which are thought to contribute to fish kills (Baden *et al.* 1993). Both toxins are heat- and acid-stable and therefore are not destroyed in the cooking process (Baden *et al.* 1993). *Karenia brevis* blooms lead to NSP poisoning in humans upon consumption of contaminated shellfish, but the aerosols created by cell lysis through beach wave action can adversely affect the respiratory system of beach goers with many people suffering asthma-like symptoms. Toxins of *K. brevis* are also responsible for vast fish kills; in particular endangered manatees in Florida have been severely affected.

Karenia brevis was initially believed to be restricted in its distribution to the tropical and subtropical regions of the Gulf of Mexico and Caribbean (Tester *et al.* 1991, Tester & Steidinger 1997), because it was assumed that the orgnanism would be unable to acclimate to lower temperatures. To date, however, *K. brevis*-associated outbreaks of NSP have also been recorded for Texas, Mississippi, Louisiana and the East coast of Florida up to North Carolina (Tester *et al.* 1991), suggesting that the organism is capable of surviving and proliferating in a broad range of temperatures. The 1987-1988 blooms of *K. brevis* along the coast of North- and South Carolina dissipated as seawater temperatures cooled and due to increasing wind stress (Tester *et al.* 1991). In Texas, beaches and shellfish beds were closed to protect human health from illnesses derived from toxic ocean spray and consumption of contaminated seafood. Brevetoxins have recently also been identified in New Zealand cockles and oysters, but the causative agent was not identified (Ishida *et al*. 1996).

Economic impacts of *K. brevis* blooms are huge. It is estimated that the cost of a bloom in the early 1970s which affected several councils in Florida was $15-20 million US dollars, and $25 million for the 1987-1988 bloom in North Carolina (Boesch *et al.* 1997), which lasted several months. These days, economic impacts are likely to be much higher as frequent *K. brevis* outbreaks, almost yearly in Florida, have severe consequences for Florida's tourism industry and fisheries.

Paralytic Shellfish Poisoning (PSP)

PSP is caused by saxitoxin and saxitoxin derivatives found in a number of different *Alexandrium* species, *Gymnodinium catenatum* and *Pyrodinium bahamense* var. *compressum* (Daranas *et al*. 2001). The illness is caused by the consumption of filter-feeding molluscs which filter these species from the water column. PSP can be fatal in some cases due to muscular and respiratory paralysis (Hallegraeff 1995) and many areas where the organisms are found and cause blooms are extensively monitored. PSP toxins cause tingling and prickly sensations, headaches, dizziness, nausea, vomiting and diarrhea. These toxins also cause mortality in fish, marine mammals and seabirds (Pitcher & Calder 2000, Doucette *et al.* 2006).

It is currently unknown whether toxin production is controlled by the dinoflagellates directly or by their symbiotic bacteria. Kodama *et al*. (1990) found that cultures of the bacterium *Moxarella* sp., isolated from *Alexandrium tamarense* produced PSP toxins, suggesting that they may indeed be of bacterial origin.

Alexandrium is one of the most harmful genera of saxitoxin-producing microalgae due to its extensive geographical distribution and the large number of toxic species (Usup *et al*. 2002). Until the late 1970s, blooms of saxitoxin-producing dinoflagellates were restricted to temperate coastal waters (Dale & Yentsch 1978). However, it appears that the ranges of *Alexandrium* spp. have since expanded and harmful blooms have been well documented in Australia, Papua New Guinea, the Philippines, Thailand, India and South Africa (Hallegraeff 1995). The tropical dinoflagellate *Pyrodinium bahamense* var. *compressum* is responsible for a greater number of human poisonings than the genus *Alexandrium* (Azanza & Taylor 2001). The former was not recorded in South East Asian waters until the 1970s. Since its establishment there, this organism has caused enormous economic and human health problems. In the Philippines alone, 1995 incidents occurred between 1983 and 1999 – 117 of which were fatal (Azanza & Taylor 2001). The GBRWHA area should be monitored for the presence of this species, as it appears to be highly invasive and has the potential to cause severe regional economic losses and health issues.

Diarrhetic Shellfish Poisoning (DSP)

DSP is primarily caused by the toxin okadaic acid and its derivatives (González *et al*. 2001). These are a group of polyether toxins which are lipophilic and cause protein phosphorylation (Daranas *et al.* 2001). Symptoms of DSP are gastrointestinal such as diarrhoea, nausea, vomiting and abdominal pains (Lange *et al*. 1990). Although no human fatalities have been recorded (Hallegraeff 1995), it is thought that these toxins are tumour promoters *(Daranas et al*. 2001), causing chronic health problems. This illness was first documented in Japan in 1976, (Yasumoto *et al.* 1980) and has mainly been reported from temperate regions (Hallegraeff 1995), although accumulation of DSP toxins in shellfish has also been recorded in Singapore and the Philippines (Holmes *et al.* 1999, Marasigan *et al.* 2001).

DSP is caused by consumption of filter feeding molluscs such as scallops and mussels (Yasumoto *et al.* 1980, Holmes *et al.* 1999). In the first recorded incidence of DSP, *Dinophysis fortii* was discovered to be the causative agent (Yasumoto *et al*. 1980). Since the initial discovery, several species of *Dinophysis* and the epibenthic *Prorocentrum*, which co-occurs with *G. toxicus* on macroalgae but is also present in the plankton (Garrard pers. obs.), are now known to cause DSP (Daranas *et al.* 2001). It was originally assumed that okadaic acid was produced directly by DSP-causing dinoflagellates, but it now appears likely that okadaic acid may also be a product derived from sulphated precursors such as dinophysistoxins in the digestive tracts of shellfish (Quilliam & Ross 1996).

Dinoflagellates from the genus *Dinophysis* can form toxic red tides with reported cell densities of up to 0.5×10^6 cells L^{-1} (Subba Rao *et al.* 1993). Species of *Dinophysis* are phagotrophic (Koike *et al*. 2000), but contain chloroplasts and several species occur in the GBRWHA within the plankton and associated with macroalgae (Heimann pers. obs). *Dinophysis* blooms typically contain other phytoplankton species (Subba Rao *et al.* 1993), which has cast doubt on *Dinophysis* as the sole causative agent of DSP. Most attempts at culture establishment have been unsuccessful (Maestrini *et al.* 1995). The first successful attempt was by Park *et al.* (2006), who cultured *Dinophysis acuminata* by feeding it the ciliate prey *Myrionecta rubra*. Hopefully, this breakthrough will enable direct quantification of the DSP toxin.

Amnesiac Shellfish Poisoning (ASP)

ASP is typically caused by the toxin domoic acid, which is produced by species of the diatom genus *Pseudo-nitzschia* and affects humans by ingestion of contaminated shellfish. ASP is characterised by both gastrointestinal and neurological symptoms, such as nausea, vomiting, abdominal cramps, and diarrhea, dizziness, headache, seizures, disorientation, short-term memory loss, respiratory difficulty, and coma. Domoic acid is a water-soluble toxin that is similar to its biochemical analogues kainic acid and glutamic acid and binds at the same receptor site in the central nervous system (Novelli *et al.* 1992). Curiously, ASP victims can suffer dementia if the ASP poisoning event involved gastrointestinal, as well as neurological, symptoms.

ASP was first reported from Canada, where, by the end of 1987, 153 patients had been diagnosed with acute symptoms through consumption of contaminated shell fish. Acute ASP has been responsible for four deaths in elderly patients and nineteen hospitalisations with twelve patients being admitted to Intensive Care. The causative agent in these poisonings was *Pseudo-nitzschia pungens*. Since then, *Pseudo-nitzschia multiseries* and *P. delicatissima* have been associated with domoic acid accumulation in mussels from France (Amzil *et al*. 2001), Japan (*P. multiseries*, (Kotaki *et al.* 1999), in Massachusetts (*P. pungens f. multiseries*, (Villareal *et al*. 1993)), the West coast of the United States (*P. australis*, (Villac *et al*. 1993)) and New Zealand (*P. multistirata* and *P. delicatissima*, (Rhodes & Scholin 2000)). By and large, domoic acid-producing species of the genus *Pseudo-nitzschia* appear to be absent from tropical locations, but a note exists from the Intergovernmental Oceanographic Commission (IOC) for subtropical and tropical (Thailand) occurrences of *P. multistriata*, which could be of concern to the GBRWHA.

Apparent Range Expansions by Toxic Microalgae

Climate change is a growing problem and microalgal communities will be affected by climatic stressors such as changes in water temperatures, ocean chemistry, ultraviolet radiation and nutrient enrichment. Indirect effects of changing oceanographic processes affecting mixing and advection have also been predicted to change microalgal communities (McKinnon *et al.* 2007). Shifts in community composition may favour toxic microalgal species.

Coral reefs are subject to frequent disturbances such as high temperatures and irradiance, crown-of-thorns outbreaks and cyclones, causing coral mortality and a reduction in coral cover. Especially in areas where eutrophication and overfishing occurs, this may cause a phase shift from coral dominated to macroalgae dominated reefs (McCook 1999). Terrestrial discharge onto the Great Barrier Reef includes sediment, fertilisers, pesticides and herbicides and other contaminants. Aerial mapping has documented plume extensions of up to fifty kilometres from the coast (Devlin & Brodie 2005). As coral bleaching increases, so does macroalgal cover on the reef (Shulman & Robertson 1996), which is likely to enhance proliferation of macroalgal-associated microalgae. On average, Carlson & Tindall (1985) found that 76% of macroalgal-associated dinoflagellate communities were toxic.

The distinction between introduced and invasive species is often not clearly made (Falk-Petersen *et al.* 2006). Introduced species are defined as species that are not indigenous to a given area, and instead have been accidentally or deliberately transported to this new location by human activity (Binggeli 1996), whilst invasive species are defined as species that establish a new range in which they proliferate, persist and spread to become economically or ecologically harmful (Mack *et al*. 2000). Although many macroalgal-associated dinoflagellates are known to be native to the GBR region, they should be classed as invasive if a range expansion has occurred (Mack *et al*. 2000). Evidence of translocation of dinoflagellates within Australia is convincing. Introduction of a dinoflagellate species is

often deduced by its absence from sediment cores and plankton records, and when sudden proliferations of the organism occur in the area. Molecular methods are also used to determine the source population of a species (see Introduction to molecular tools section) (Hallegraeff 1992, Bolch & de Salas 2007). Likely reasons for the increased geographical range of species are the transport to non-native locations via ships' ballast water, aquaculture and increased tourism. It is estimated that up to ten thousand species are transported in ballast water each day (Carlton 1999), taken up from native ports and released upon arriving at the destination. As ocean travel increases, so does the distribution of marine microalgae across the globe. Many species of dinoflagellates are able to survive in ballast water by forming cysts or using modes of nutrition other than photosynthesis (Doblin *et al.* 2004).

The ABWMAC schedule of introduced species has listed *Alexandrium minutum*, *A. catenella*, *A. tamarense* and *Gymnodinium catenatum* as target pest species (Table 1), which are likely to be spread through ballast water transport. These species have a large global distribution and their cysts have been detected in the ballast waters of ships in Australian ports and successfully germinated (Hallegraeff & Bolch 1992, Hallegraeff 1998). The phenomenon of toxic algal blooms causing shellfish poisonings, was virtually unknown in Australia until 1986, when large concentrations of saxitoxin were discovered in wild mussels in Port Phillip Bay and saxitoxin derivatives, thought to be produced by *Alexandrium catenella* and *Gymnodinium catenatum* respectively, in shellfish farms in Tasmania (Hallegraeff 1992). As *Alexandrium* spp. and *Gymnodinium* spp. have not been found in cyst records from sediments or previous plankton monitoring schemes in Australia, it is likely that they have been introduced (Hallegraeff 1992). The first verification of a toxic algal outbreak in New Zealand occurred in 1993 for the PSP-causing toxic dinoflagellate *Alexandrium minutum* in the Bay of Plenty (Chang *et al.* 1997). Investigation into the toxin profiles of cockles and oysters during this event also discovered evidence of brevetoxin contamination (Ishida *et al.* 1996) and the co-occurrence of the neurotoxin-producer *Karenia breviculata* (Chang *et al.* 2001). Subsequent outbreaks have occurred with considerable costs to the shellfish industry, leading to the initiation of extensive monitoring programs to aid in the early detection of toxic microalgal blooms (Rhodes *et al.* 2001).

Distribution of Toxic Microalgae within the GBR and Australasia

Very little is known about community structure, distribution and abundance of sediment- and macroalgal-associated microalgae within the GBR ecosystem. Sites of surveys within Queensland and locations of known blooms of toxic microalgae within the Australasian area are presented in Figure 1. Gillespie *et al*. (1985) investigated a range of sites across Queensland, but restricted the investigation to the distribution of *Gambierdiscus toxicus*, although the presence of the genera *Ostreopsis* and *Prorocentrum* were noted. Queensland ports have been surveyed sporadically as part of the Port Baseline Surveys for Introduced Marine Pests coordinated by CSIRO-CRIMP, e.g. Hay Point/ Mackay, 17 May to 1 June 1997 (CSIRO 1998) and the Port of Townsville, November 2000 (Neil et al. 2001). Survey follow-up included morphological identification of toxic microalgae from within the water column and sediments. Cysts of the genus *Gymnodinium* were recorded although none of the ABWMAC target pest microalgal species (Table 1) were found.

Species producing paralytic shellfish toxins, such as *Alexandrium catenella*, *A. minutum* and *Gymnodinium catenatum*, are now known to cause blooms in southern Australia (Hallegraeff *et al.* 1988) and *G. catenatum* has caused large economic losses in New Zealand (Rhodes *et al.* 2001). *Gymnodinium catenatum* has also been found in the tropical plankton assemblages of Singapore (Holmes & Teo 2002), suggesting that although it is commonly

only found in temperate areas it can survive and grow in a wide range of seawater temperatures. Although *Dinophysis caudata* occurred in low numbers in Singapore, the green mussel *Perna viridis*, which feeds upon this species, contained persistent, low concentrations of diarrhetic shellfish toxins. *Dinophysis caudata* is also abundant in tropical phytoplankton communities of the GBR and combined with the recent introduction of *P. viridis* to the GBRWHA, DSP outbreaks could increase in this region.

The saxitoxin-producing dinoflagellate *Pyrodinium bahamense* var. *compressum* is the greatest threat to countries within South East Asia. Although it has only caused bloom events since the 1970s, there have been a large number of fatalities from PSP events and bloom events now occur in the Philippines, Malaysia, Indonesia, Jakarta, Brunei and Papua New Guinea (Maclean 1977, 1989, Matsuoka *et al*. 1998, Azanza & Taylor 2001).

Assessment of Toxic Microalgal Species of Risk in the GBR Ecosystem

The target pest species outlined in the ABWMAC schedule of introduced species commonly form blooms in temperate regions and may currently not be considered of great concern in Queensland. Range expansions of tropical macro- (e.g. *Caulerpa taxifolia* (Meinesz *et al*. 1993, Chisholm *et al*. 2000, Dalton 2000, Meinesz *et al.* 2001, Fama *et al*. 2002)) and toxic microalgae (e.g. *Karenia brevis* Florida to Texas, (Tester *et al*. 1991)) to more subtropical and even temperate locations have been demonstrated. This indicates that the development of cold-tolerant strains is possible for both macro- and microalgae. Although no strong case can be made at present for or against the potential for more temperate species of microalgae to adapt to tropical conditions, many toxic dinoflagellates show increased toxin production and growth at higher temperatures between 22-25°C. Furthermore, some of the most potent toxic microalgae already occur in the GBRWHA, e.g. cyst records of *Pyrodinium bahamense* var. *compressum*, *Gambierdiscus toxicus*, *Coolia* spp. and *Ostreopsis* spp., area and in tropical regions and those species and strains must be monitored for their occurrence, abundance and distribution pattern in the GBR to protect human health and biodiversity.

The ABWMAC target pest species have historically caused economic problems in temperate regions such as California, Alaska and Japan (Hallegraeff *et al.* 1988), and recently in Australia. Cysts of *Alexandrium catenella* are now common around the coastline of New South Wales, where coastal temperatures range from 13-25°C (Hallegraeff *et al.* 1998). An increase in temperature from 17-25°C, caused increased germination success in Australian isolates of *A. catenella* (Hallegraeff *et al.* 1998), which is within sea temperatures for Queensland. Ballast water from a single ship in Eden, New South Wales, Australia was estimated to contain over three hundred million *Alexandrium* cysts (Hallegraeff & Bolch 1992). It would only be necessary for a few cells to acclimatise to the seawater temperatures in this region and successfully germinate to establish a bloom. Toxic dinoflagellate cysts are likely to accumulate in sheltered areas with soft, silty bottoms (CSIRO 1998), fitting the description of the habitat within many ports (Garrard pers obs). If ballast water introductions occur in this area, cysts may remain viable in the sediment for five to ten years (Anderson *et al.* 1995).

Although they are not on the list of target species, *Alexandrium tamiyavanichii* and *Pyrodiumium bahamense* var *compressum* are saxitoxin producers, which are known to cause PSP in tropical waters (Usup *et al.* 2002). The latter species was previously known for its bioluminescent blooms in the Caribbean (Seliger *et al.* 1970), and has spread through South East Asia causing numerous cases of human poisonings, a number of which have resulted in fatalities (Azanza & Taylor 2001). Although to date no blooms of this species have occurred in Australian waters, cysts have been found in the fossil record, suggesting

that *P*. *bahamense* var. *compressum* may be common in this area but for unknown reasons presently does not proliferate. With climate change, many species are extending their geographic range south and blooms of *P. bahamense* var *compressum* have been linked to El Niño events (Maclean 1989). The possibility of ballast water transport of this cyst-forming species from other areas, or transport via currents from Papua New Guinea is realistic and there is every possibility of this species spreading to Northern Australia (Maclean 1989).

Knowledge of how climate change will affect microalgal community structure is limited and fast, efficient and cost-effective methods of detecting toxic microalgae are necessary in order to provide early warning and prevent economic losses and risk to human health. Prior to 1993, *Heterosigma akashiwo* was the only toxic dinoflagellate forming blooms in New Zealand, which, although causing massive fish kills, did not pose a risk to human health. Since then, a large number of toxic microalgal blooms have occurred and nearly all known toxic microalgal species occur in New Zealand now (Rhodes *et al.* 2001). Microalgal monitoring programmes in New Zealand involve weekly surveys and have been internationally accredited (Rhodes *et al*. 2001). In contrast, in Queensland only a few one-off, morphologically-based surveys have been carried out.

In terms of human illness caused by the consumption of seafood contaminated with microalgal toxins, ciguatera fish poisoning has been the greatest threat to residents in the GBR region. The earliest reports were by Captain Cook in the 18th Century (Lewis 2006). In Australia, ciguatera poisoning is correlated to consumption of fish caught in Queensland and the Northern Territory (Capra & Cameron 1991), with most cases being linked to consumption of fish caught on the GBR (Lewis 2006). Approximately 0.16 cases per 10,000 residents are reported in Queensland per annum, although it is estimated that fewer than 20% of cases that occur are reported and/or diagnosed (Lewis 2001). Between 1964 and 1974, 750 cases of ciguatera were reported in Townsville alone (Gillespie *et al.* 1986). This is not to say that this is the only risk posed by toxic microalgae. As explained above, many species originally only occurred in the northern hemisphere but are now being detected in the southern hemisphere. Similarly, temperate species have begun to expand their range into tropical and subtropical regions. Our knowledge of toxic microalgal species composition in the GBR is extremely limited and it is therefore impossible to determine which species pose the greatest risk in this region. Equally, the limited knowledge of microalgal flora of the GBR region makes it impossible to determine introductions of microalgal species from other regions via aquaculture, tourism and/or ballast water and range expansions of species already native to this area. In the light of climate change, it is mandatory now to establish monitoring of planktonic and benthic species and to develop regionally useful molecular methods in order to provide an early warning system for the presence and distribution of these organisms.

Introduction to Molecular Tools Suitable for the Detection of Toxic Microalgae in the GBR

Prior to the development of molecular techniques, microalgae were solely identified to species level based on their morphological characteristics. Morphological techniques are time consuming and expensive (manpower) and require considerable taxonomical expertise. However, misidentification of some morphologically similar species can never be completely excluded. Many toxic and non-toxic species from the genus *Alexandrium* are morphologically very similar (Balech 1995). The degree of morphological plasticity in response to environmental factors (Lilly *et al.* 2005) is also presently unknown leading to the debate whether *Alexandrium tamarense*, *A. catenella* and *A*. *fundyense* (the "*tamarensis* complex") are distinct species or different strains of the same species.

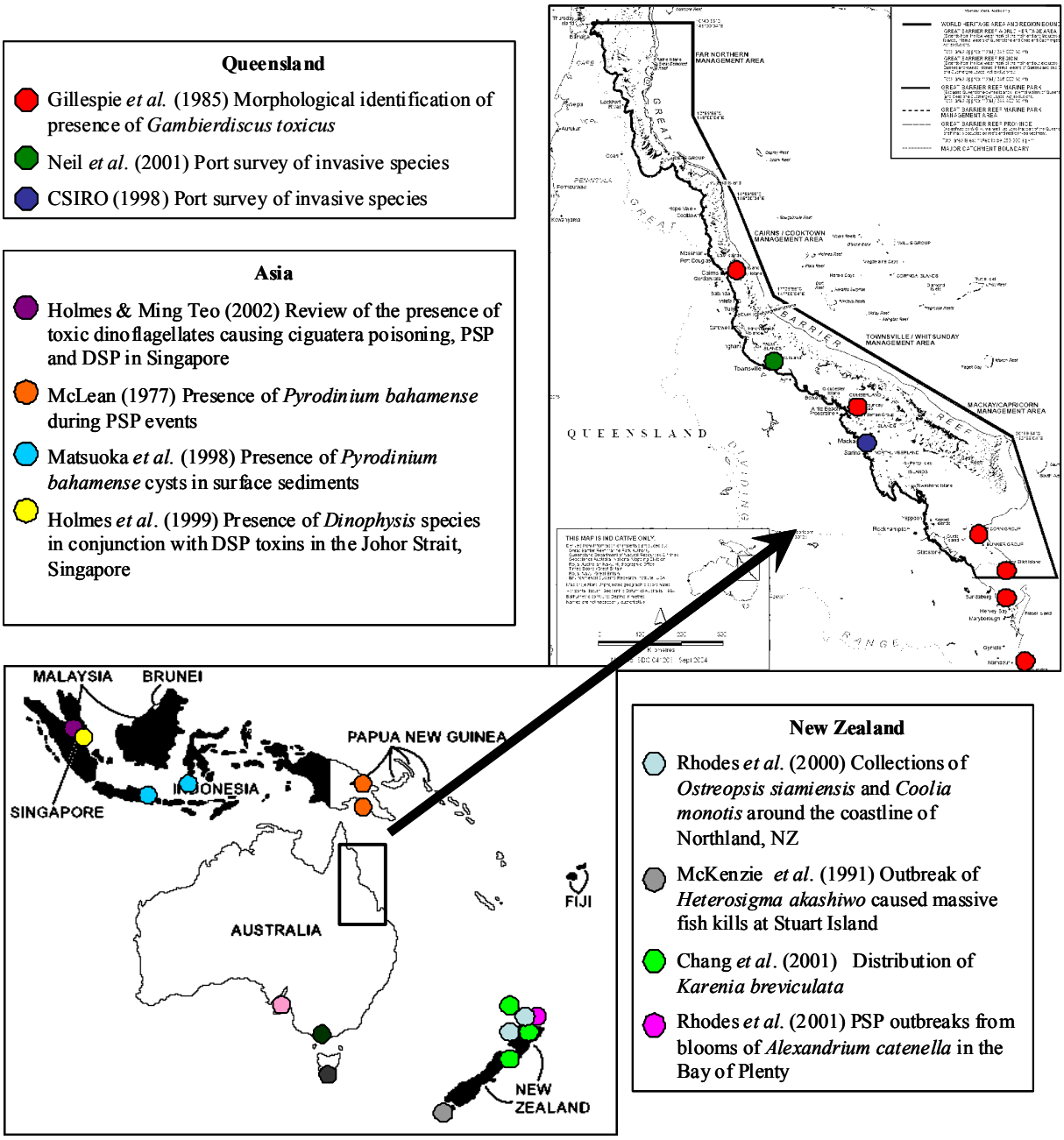


Figure 1. Map of the distribution of toxic microalgal species in Australasia identified from poisoning events and blooms and areas where toxic microalgal surveys have taken place within the GBR ecosystem

Figure 1: Known distributions of toxic microalgae and survey locations for Queensland, Australasia and New Zealand.

Molecular analyses of the large and small subunit ribosomal RNA genes established that genetic variation correlated with geographically distinct groups (Scholin & Anderson 1994, Scholin *et al.* 1994) but not with morphotypically defined species, which suggests that phenotypic plasticity exists. Many *Alexandrium* cysts cannot be identified to species level based on morphology (Kamikawa *et al.* 2005, Kamikawa *et al.* 2007). These problems clearly demonstrate that morphological techniques alone are ineffective to protect human health, unless they are supported by genetic identification of toxic microalgae.

Molecular methods are fast becoming a popular tool for the rapid assessment of the presence of microscopic organisms. Most detection methods include nucleic acid hybridization, where complementary strands of DNA or RNA bind to specific nucleotide sequences. This is the basis for many methods including the polymerase chain reaction (PCR), fluorescent *in situ* hybridization (FISH), sandwich hybridization assays and many more. Open access, publicly available nucleotide sequences and the ability to compare and align sequences has lead to the development of species-specific primers or synthetic oligonucleotide probes for detection. The majority of molecular detection methods target nuclear and plastid ribosomal genes as sequences for many species are available and these genes contain conserved regions for annealing of PCR primers as well as variable regions for species identification (Maidak *et al*. 2000, Groben *et al*. 2004)

Polymerase Chain Reaction (PCR)

The application of PCR with species-specific primers is the most straight-forward method for detection of a particular strain of microalga in environmental samples. Only samples positive for the target sequence produce reaction products, which are visualised by ethidium bromide staining and gel electrophoresis. Godhe *et al*. (2001) used this method to detect two species of toxic dinoflagellates from within the plankton (*Gymnodinium mikimotoi* and *Alexandrium minutum*). Levels of detection, however, were restricted by organismal abundance as a sample containing 115 cells of a target species yielded negative results. Patil *et al.* (2005) performed a two-step nested PCR using universal primers to amplify a portion of the large subunit ribosomal RNA gene first, followed by species-specific primers to amplify a variable region within this portion. This boosted the level of detection from environmental samples, giving successful readings for samples with ≥5 cells. This method also successfully detected ≥5 cysts from spiked ballast water samples. The same method was also used to detect cysts of *Scrippsiella trochoidea*, *Protoceratium reticulatum* and *Lingulodinium polyedrum* in sediment samples (Godhe *et al*. 2002). This is a good result as detection of microalgae from sediments often may not be as successful as from the water column due to sediments containing inhibitors for PCR (Kamikawa *et al*. 2005).

Real-time polymerase chain reaction (RT-PCR) is an adaptation of PCR. In RT-PCR the quantity of the sequence amplified is fluorescently measured after each cycle, not only allowing detection but also quantification of the target species. As well as the two PCR primers, a flourogenic oligonucleotide probe, carrying a fluorescent indicator at the 5' end and a quencher dye at the 3' end, is used. This hybridises with the target sequence between the annealing sites of the two primers. *Taq* DNA polymerase with 5'- 3' exonuclease activity degrades the probe during the PCR, giving out a quantifiable signal (Holland *et al*. 1991; Lee *et al.* 1993) as the probe is degraded separating the indicator from the quencher. This method has been shown to be successful at quantifying toxic dinoflagellates from the water column (Hosoi-Tanabe & Sako 2005). CTAB DNA extraction (Zhou *et al.* 1999) in conjunction with RT-PCR is suitable for the quantification of harmful algal cysts from environmental samples (Kamikawa *et al.* 2005). Kamikiwa *et al.* (2007) suggested that this is a faster and more reproducible method than the nested PCR method developed by Godhe *et al.* (2002) as it only contains one step.

Fluorescence in situ Hybridization (FISH)

FISH is a method designed to detect intact microbial cells in environmental samples (Amann *et al*. 2001). A fluorescently labelled probe is designed to detect a specific target nucleic acid sequence (DeLong *et al.* 1989). Originally this method was designed to detect prokaryotic cells which could not be cultured (Amann *et al.* 1990). It appears that FISH may also be a suitable technique for detection of some species of toxic microalgae (Tyrrell *et al*. 1997, Sako *et al.* 2004, Hosoi-Tanabe & Sako 2006). FISH typically involves four steps (Amann *et al.* 2001). A sample is fixed first to preserve the cells and to increase cell wall permeability for probe entry (Tyrrell *et al.* 1997). The probe then hybridizes with the target nucleic acid. Washing removes excess probe and quantification is done by epiflourescence microscopy.

Generally the oligonucleotide probes are designed to target ribosomal RNA (transcribed from ribosomal RNA genes) as there are large numbers of copies within the cell giving a greater signal (Sako *et al.* 2004). However, probes targeting the ribosomal RNA genes themselves may provide a better idea of the number of cells present in a sample as the number of these genes remains constant in the genome during different physiological states (DeLong *et al.* 1989, Adachi *et al*. 1996). Probes against ribosomal RNA were developed for *Alexandrium tamarense* and *A. catenella*. These proved to be specific, rapid, cost-effective and sensitive enough to detect small numbers or organisms present in environmental samples (Sako *et al.* 2004, Hosoi-Tanabe & Sako 2006). Identification time of toxic marine microalgae was reduced to 30 min (Hosoi-Tanabe & Sako 2006) from approximately three hours (Tyrrell *et al.* 1997) prior to the development of these probes. Although this method was successful for identification of these species, Sako *et al.* (2004) suggested that it may not be suitable for microalgae with soft cell walls, as cells are required to remain intact throughout this process. As this method requires detection of ribosomal RNA, it is not likely to be suitable for determination of dormant cysts within the sediment, which will not contain large quantities of RNA.

Sandwich Hybridization

Sandwich hybridization assays require the use two of synthetic oligonucleotide probes. The capture probe is biotinylated and attached to a strepavidin-coated solid support, such as the surface of tissue plate wells (Tyrrell *et al*. 2002, Jones *et al*. 2007, Diercks *et al*. *in press*). The second probe is a reporter probe which carries either a fluorescent, colourimetric or chemiluminescent signal (Tyrrell *et al.* 1997). The capture probe immobilises the target nucleic acid if present in the sample and the reporter probe is added. The latter binds to a different portion of the target. Excess (unbound) reporter probe is removed by washing and bound (hybridised) reporter probe is detected directly by fluorescence, if the reporter probe is fluorescent (Ahn *et al.* 2006), or a substrate is added causing a colourimetric or chemiluminescent signal when it reacts with the enzyme-tagged reporter probe (Tyrrell *et al.* 1997, Tyrrell *et al*. 2002, Jones *et al.* 2007, Diercks *et al*. *in press*). Hence detection is only achieved when the nucleic acids have regions complementary to both probes (Tyrrell *et al.* 1997).

References

Adachi M, Sako Y, Ishida Y. (1996) Identification of the toxic dinoflagellates *Alexandrium catenella* and *A. tamarense* (Dinophyceae) using DNA probes and whole-cell hybridization. *Journal of Phycology* 32: 1049-1052.

Ahn S, Kulis D.M, Erdner D.L, Anderson D.M, Walt D.R. (2006) Fiber-optic microarray for simultaneous detection of multiple harmful algal bloom species. *Applied and Environmental Microbiology* 72: 5742-5749.

Amann R, Fuchs B.M, Behrens S. (2001) The identification of microorganisms by fluorescence *in situ* hybridisation. *Current Opinion in Biotechnology* 12: 231-236.

Amann R.I, Krumholz L, Stahl D.A. (1990) Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. *Journal of Bacteriology* 172: 762-770.

Amzil Z, Fresnel J, le Gal D, Billard C. (2001) Domoic acid accumulation in French shellfish in relation to toxic species of *Pseudo-nitzschia multiseries* and *P. pseudodelicatissima* . *Toxicon* 39: 1245-1251.

Anderson D.M, Fukuyo Y, Matsuoka K. (1995) Cyst methodologies. In: Hallegraeff G.M, Anderson D.M, Cembella A.D. (eds) Manual on Harmful Marine Microalgae. UNESCO, Paris, France.

Azanza R.V, Taylor F.J.R.N. (2001) Are *Pyrodinium* blooms in the Southeast Asian region recurring and spreading? A View at the End of the Millennium. *Ambio* 30: 356-364.

Baden DG, Reins KS, Gawley RE, Jeglitsch G, Adams D. (1993) The a-ring lactone of brevetoxin PbTx-3 is required for sodium channel orphan receptor binding and activity. Natural Toxins.

Bagnis R, Bennett J, Barsinas M. (1985) Epidemiology of ciguatera in French Polynesia from 1960-1984. In: Gabrie C, Salvat B. (eds) Proceedings of the Fifth International Coral Reef Conference, Tahiti, p 475-482.

Bagnis R, Legrand A.P, Inoue A. (1990) Follow-up of a bloom of the toxic dinoflagellate *Gambierdiscus toxicus* on a fringing reef in Tahiti. In: Graneli E, Sundstrom B, Edler L, Anderson D.M. (eds) Toxic Marine Phytoplankton. Elsevier Science, New York, p 99-103.

Balech E. (1995) The Genus *Alexandrium halim* (Dinoflagellata), Vol. Sherwin Island Press, Cork, Ireland.

Binggeli P. (1996) A taxonomic, biogeographical and ecological overview of invasive woody plants. *Journal of Vegetation Science* 7: 121-124.

Boesch D.F, Anderson D.A, Horner R.A, Shumway S.E, Tester P.A, Whitledge T.E. (1997) Harmful algal blooms in coastal waters: options for prevention, control and mitigation. NOAA Coastal Ocean Program, Decision Analysis Series No. 10, Special Joint Report with the National Fish and Wildlife Foundation, February 1997.

Bolch C.J.S, de Salas M.F. (2007) A review of the molecular evidence for ballast water introduction of the toxic dinoflagellates *Gymnodinium catenatum* and the *Alexandrium* "*tamarensis* complex" to Australasia. *Harmful Algae* 6: 465-485.

Bomber J.W, Guillard R.R.L, Nelson W.G. (1988) Roles of temperature, salinity, and light in seasonality, growth, and toxicity of ciguatera-causing *Gambierdiscus toxicus* Adachi et Fukuyo (Dinophyceae). *Journal of Experimental Marine Biology and Ecology* 115: 53-65.

Bouligand Y, Norris V. (2001) Chromosome separation and segregation in dinoflagellates andbacteria may depend on liquid crystalline states. *Biochimie* 83: 187-192.

Burkholder J.M, Noga E.J, Hobbs C.H, Glasgow H.B. (1992) New phantom dinoflagellate is the causative agent of major estuarine fish kills (Nature, Vol 358, Pg 407, 1992). *Nature* 360: 768-768.

Calvert G.M. (1991) The recognition and management of ciguatera fish poisoning. In: Miller DM (ed) Ciguatera Seafood Toxins. CRC Press, Boca Raton, Florida.

Capra M.F, Cameron J. (1991) Ciguatera in Australia. In: Miller DM (ed) Ciguatera Seafood Toxins. CRC Press, Florida.

Carlton J.T. (1999) The scale and ecological consequences of biological invasions in the world's oceans. In: Sunderland O.J, Schei P.J, Viken A (eds) Invasive species and biodiversity. Kluwer Academic Publishers, Dordrecht, p 195-212.

Chang F.H, Anderson D.M, Kulis D.M, Till D.G. (1997) Toxin production of *Alexandrium minutum* (Dinophyceae) from the Bay of Plenty, New Zealand. *Toxicon* 35: 393-409.

Chateau-Degat M-L, Chinain M, Cerf N, Gingras S, Hubert B, Dewailly E. (2005) Seawater temperature, *Gambierdiscus* spp. variability and incidence of ciguatera poisoning in French Polynesia. *Harmful Algae* 4: 1053-1062.

Chinain M, Faust M.A, Pauillac S. (1999) Morphology and molecular analyses of three toxic species of *Gambierdiscus* (Dinophyceae): *G. pacificus*, sp nov., *G. australes*, sp nov., and *G. polynesiensis*, sp nov. *Journal of Phycology* 35: 1282-1296.

Chisholm J.R.M, Marchioretti M, Jaubert J.M. (2000) Effect of low water temperature on metabolism and growth of a subtropical strain of *Caulerpa taxifolia* (Chlorophyta). *Marine Ecology-Progress Series* 201: 189-198.

CSIRO (1998) Introduced species survey Port of Hay Point, Queensland. Centre for Research on Introduced Marine Pests.

Dale B, Yentsch C.M. (1978) Red tide and paralytic shellfish poisoning. *Oceanus* 21: 41-49.

Dalton R. (2000) Researchers criticize response to killer algae. *Nature* 406: 447-447.

Daranas A.H, Norte M, Fernandez J.J. (2001) Toxic marine microalgae. *Toxicon* 39: 1101-1132.

DeLong E.F, Wickham G.S, Pace N.R. (1989) Phylogenetic stains: ribosomal RNA-based probes for the identification of single cells. *Science* 243: 1360-1363.

Devlin MJ, Brodie J. (2005) Terrestrial discharge into the Great Barrier Reef Lagoon: nutrient behavior in coastal waters. *Marine Pollution Bulletin* 51: 9-22.

Diercks S, Medlin L.K, Metfies K. (*in press*) Colorimetric detection of the toxic dinoflagellate *Alexandrium minutum* using sandwich hybridization in a microtiter plate assay. Harmful Algae In Press, Corrected Proof.

Doblin M.A, Popels L.C, Coyne K.J, Hutchins D.A, Cary S.C, Dobbs F.C. (2004) Transport of the harmful bloom alga *Aureococcus anophagefferens* by oceangoing ships and coastal boats. *Applied and Environmental Microbiology* 70: 6495-6500.

Dodge J.D. (1966) The Dinophyceae. In: Godward MBE (ed) The Chromosomes of the Algae. Edward Arnold Ltd, London, p 96-115.

Doucette G.J, Cembella A.D, Martin J.L, Michaud J, Cole T.V.N, Rolland R.M. (2006) Paralytic shellfish poisoning (PSP) toxins in North Atlantic right whales *Eubalaena glacialis* and their zooplankton prey in the Bay of Fundy. *Canada Marine Ecology Progress Series* 306: 303-313.

Edler L, Olsson P. (1985) Observations on diel migration of *Ceratium furca* and *Prorocentrum micans* in a stratified bay on the Swedish west coast. In: Anderson D.M, White A.W, Baden D.G (eds) Toxic Dinoflagellates. Elsevier, New York, p 195-2000.

Falk-Petersen J, Bohn T, Sandlund O.T. (2006) On the numerous concepts in invasion biology. *Biological Invasions* 8: 1409-1424.

Fama P, Jousson O, Zaninetti L, Meinesz A, Dini F, Di Giuseppe G, Millar A.J.K, Pawlowski J. (2002) Genetic polymorphism in *Caulerpa taxifolia* (Ulvophyceae) chloroplast DNA revealed by a PCR-based assay of the invasive Mediterranean strain. *Journal of Evolutionary Biology* 15: 618-624.

Furnas M.J, Mitchell A.W. (1996) Biological oceanography of the Great Barrier Reef. In: Anon (ed) Proceedings: the Great Barrier Reef, Science, Use and Management. Great Barrier Reef Marine Park Authority, Townsville, p 75-87.

Gaines G, Elbrächter M. (1987) Heterotrophic nutrition. In: Taylor F.J.R (ed) The Biology of Dinoflagellates. Blackwell, Oxford.

Garces E, Maso M, Camp J. (2002) Role of temporary cysts in the population dynamics of *Alexandrium taylori* (Dinophyceae). *Journal of Plankton Research* 24: 681-686.

Gillespie N.C, Holmes M.J, Burke J.B, Doley J. (1985) Distribution and periodicity of *Gambierdiscus toxicus* in Queensland, Australia. In: Anderson DM, White AW, Baden DG (eds) Toxic Dinoflagellates. Elsevier, New York.

Gillespie N.C, Lewis R.J, Pearn J.H, Bourke A.T.C, Holmes M.J, Bourke J.B, Shields W.J. (1986) Ciguatera in Australia - occurrence, clinical-features, pathophysiology and management. *Medical Journal of Australia* 145: 584-590.

Godhe A, Otta S.K, Rehnstam-Holm A-S, Karunasagar I, Karunasagar I. (2001) Polymerase chain reaction in detection of *Gymnodinium mikimotoi* and *Alexandrium minutum* in field samples from Southwest India. *Marine Biotechnology* 3: 152-162.

Godhe A, Rehnstam-Holm A-S, Karunasagar I, Karunasagar I. (2002) PCR detection of dinoflagellate cysts in field sediment samples from tropic and temperate environments. *Harmful Algae* 1: 361-373.

González J, Vieytes M, Vieites J, Botana L. (2001) Solubility of the diarrheic shellfish toxin okadaic acid in supercritical CO_2. *Journal of the American Oil Chemists' Society* 78: 77-81.

Groben R, John U, Eller G, Lange M, Medlin L.K. (2004) Using fluorescently-labelled rRNA probes for hierarchical estimation of phytoplankton diversity - a mini-review. *Nova Hedwigia* 79: 313-320.

Hackett J.D, Scheetz T.E, Yoon H.S, Soares M.B, Bonaldo M.F, Casavant T.L, Bhattacharya D. (2005) Insights into a dinoflagellate genome through expressed sequence tag analysis. Bmc Genomics 6.

Hales S, Weinstein P, Woodward A (1999) Ciguatera (Fish Poisoning), El Nino, and Pacific Sea Surface Temperatures. *Ecosystem Health* 5: 20-25.

Hallegraeff G.M (1992) Harmful algal blooms in the Australian region. *Marine Pollution Bulletin* 25: 186-190.

Hallegraeff G.M. (1995) Harmful algal blooms: a global overview. In: Hallegraeff G.M, Anderson D.M, Cembella A.D (eds) Manual on Marine Harmful Microalgae. UNESCO, Paris, France.

Hallegraeff G.M. (1998) Transport of toxic dinoflagellates via ships' ballast water: bioeconomic risk assessment and efficacy of possible ballast water management strategies. *Marine Ecology-Progress Series* 168: 297-309.

Hallegraeff G.M. (2003) Harmful algal blooms: a global overview. In: Hallegraeff G.M, Anderson D.M, Cembella A.D (eds) Manual on Marine Harmful Microalgae. UNESCO, Paris, France.

Hallegraeff G.M, Bolch C.J. (1992) Transport of diatom and dinoflagellate resting spores in ships' ballast water - implications for plankton biogeography and aquaculture. *Journal of Plankton Research* 14: 1067-1084.

Hallegraeff G.M, Marshall J.A, Valentine J, Hardiman S. (1998) Short cyst-dormancy period of an Australian isolate of the toxic dinoflagellate *Alexandrium catenella*. *Marine and Freshwater Research* 49: 415-420.

Hallegraeff G.M, Steffensen D.A, Wetherbee R. (1988) 3 Estuarine Australian dinoflagellates that can produce paralytic shellfish toxins. *Journal of Plankton Research* 10: 533-541.

Harbungs M.D, Jayabalan N, Chineah V. (2001) Seasonal distribution of potentially toxic benthic dinoflagellates in the lagoon of Trou Aux Bishes, Mauritius, Albion Fisheries Research Centre, Reduit, Mauritius.

Herzog M, Soyer M.O. (1981) Distinctive features of dinoflagellate chromatin - absence of nucleosomes in a primitive species *Prorocentrum micans* E. *European Journal of Cell Biology* 23: 295-302.

Hewitt C.L, Martin R.B. (2001) Revised protocols for baseline port surveys for introduced marine species: survey design, sampling protocols and specimen handling. Centre for Research on Introduced Marine Pests, Technical Report 22, CSIRO.

Holland P.M, Abramson R.D, Watson R, Gelfand D.H. (1991) Detection of specific polymerase chain reaction product by utilizing the 5' → 3' exonuclease activity of *Thermus aquaticus* DNA polymerase. Proceedings of the National Academy of Sciences 88: 7276-7280.

Holmes M.J, Lewis R.J, Ashley A.J, Hoy W.W. (1995) Cooliatoxin, the first toxin from *Coolia monotis* (Dinophyceae). *Natural Toxins* 3: 355-362.

Holmes M.J, Lewis R.J, Poli M.A, Gillespie N.C. (1991) Strain dependent production of ciguatoxin precursors (gambiertoxins) by *Gambierdiscus toxicus* (Dinophyceae) in culture. *Toxicon* 29: 761-775.

Holmes M.J, Ming T.S, Lee F.C, Khoo H.W. (1999) Persistent low concentrations of diarrhetic shellfish toxins in green mussels *Perna viridis* from the Johor Strait, Singapore: first record of diarrhetic shellfish toxins from South-East Asia. *Marine Ecology Progress Series* 181: 257-268.

Holmes M.J, Teo S.L.M. (2002) Toxic marine dinoflagellates in Singapore waters that cause seafood poisonings. *Clinical and Experimental Pharmacology and Physiology* 29: 829-836.

Hosoi-Tanabe S, Sako Y. (2005) Rapid detection of natural cells of *Alexandrium tamarense* and *A. catenella* (Dinophyceae) by fluorescence in situ hybridization. *Harmful Algae* 4: 319-328.

Hosoi-Tanabe S, Sako Y. (2006) Development and application of fluorescence in situ hybridization (FISH) method for simple and rapid identification of the toxic dinoflagellates *Alexandrium tamarense* and *Alexandrium catenella* in cultured and natural seawater. *Fisheries Science* 72: 77-82.

International Oceanographic Commission (accessed 09-12-2007): http://www.bi.ku.dk/ioc/default.asp

Ishida H, Muramatsu N, Nukaya H, Kosuge T, Tsuji K (1996) Short communication. *Toxicon* 34: 1050-1053.

Jones W.J, Preston C.M, Marin R, Scholin C.A, Vrijenhoek R.C. (2007) A robotic molecular method for in situ detection of marine invertebrate larvae. *Molecular Ecology Notes* (Online Early Articles): doi:10.1111/j.1471-8286.2007.02021.x

Kamikawa R, Hosoi-Tanabe S, Nagai S, Itakura S, Sako Y (2005) Development of a quantification assay for the cysts of the toxic dinoflagellate *Alexandrium tamarense* using real-time polymerase chain reaction. *Fisheries Science* 71: 987-991.

Kamikawa R, Nagai S, Hosoi-Tanabe S, Itakura S, Yamaguchi M, Uchida Y, Baba T, Sako Y (2007) Application of real-time PCR assay for detection and quantification of *Alexandrium tamarense* and *Alexandrium catenella* cysts from marine sediments. *Harmful Algae* 6: 413-420.

Kodama M, Ogata T, Sakamoto S, Sato S, Honda T, Miwatani T (1990) Production of paralytic shellfish toxins by a bacterium *Moraxella* sp. isolated from *Protogonyaulax tamarensis*. *Toxicon* 28: 707-714.

Koike K, Koike K, Takagi M, Ogata T, Ishimaru T. (2000) Evidence of phagotrophy in *Dinophysis fortii* (Dinophysiales, Dinophyceae), a dinoflagellate that causes diarrhetic shellfish poisoning (DSP). *Phycological Research* 48: 121-124.

Kotaki Y, Koike K, Sato S, Ogata T, Fukuyi Y, Kodama M. (1999) Confirmation of domoic acid production of *Pseudo-nitzschia multiseries* isolated from Ofunato Bay, Japan. *Toxicon* 37: 677-682.

Lange S, Andersson G.L, Jennishe E, Lonnroth I, Li X.P, Edebo L. (1990) Okadaic acid produces drastic histopathological changes of the rat intestinal mucosa and with concomitant hypersecretion. In: Graneli E, Sundstrom B, Edler L, Anderson DM (eds) Toxic Marine Phytoplankton. Elsevier Science, New York, p 356-361.

Lee L.G, Connell C.R., Bloch W. (1993) Allelic discrimination by nick-translation PCR with fluorgenic probes. *Nucleic Acids Research* 21: 3761-3766.

Legrand A.P, Crunchet R, Bagnis M, Murata M, Ishibashi I, Yasumoto T (1990) Chromatographic and spectral evidence of multiple ciguatera toxins. In: Graneli E, Sundrom B, Edler L, Anderson D.M. (eds) Toxic Marine Phytoplankton. Elsevier, New York, p 374-378.

Lehane L, Lewis R.J. (2000) Ciguatera: recent advances but the risk remains. International *Journal of Food Microbiology* 61: 91-125.

Lenoir S, Ten-Hage L, Turquet J, Quod J-P, Bernard C, Hennion M-C. (2004) First evidence of palytoxin analogues from an *Ostreopsis mascarenensis* (Dinophyceae) benthic bloom in Southwestern Indian Ocean. *Journal of Phycology* 40: 1042-1051.

Lewis R.J. (1992) Ciguatoxins are potent ichthyotoxins. *Toxicon* 30: 207-211.

Lewis R.J. (2001) The changing face of ciguatera. *Toxicon* 39: 97-106.

Lewis R.J. (2006) Ciguatera: Australian perspectives on a global problem. *Toxicon* 48: 799-809.

Lewis R.J., Holmes M.J (1993) Origin and transfer of toxins involved in ciguatera. *Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology* 106: 615-628.

Lewis R.J, Sellin M, Poli M.A, Norton R.S, MacLeod J.K, Sheil M.M. (1991) Purification and characterization of ciguatoxins from moray eel (*Lycodontis javanicus*, Muraenidae). *Toxicon* 29: 1115-1127.

Lilly E.L, Halanych K.M, Anderson D.M. (2005) Phylogeny, biogeography, and species boundaries within the *Alexandrium minutum* group. *Harmful Algae* 4: 1004-1020.

Mack R.N, Simberloff D, Lonsdale W.M, Evans H, Clout M, Bazzaz F.A. (2000) Biotic invasions: Causes, epidemiology, global consequences, and control. *Ecological Applications* 10: 689-710.

Maclean J.L. (1977) Observations on *Pyrodinium bahamense* Plate, a Toxic Dinoflagellate, in Papua-New-Guinea. *Limnology and Oceanography* 22: 234-254.

Maclean J.L. (1989) Indo-Pacific red tides, 1985-1988. *Marine Pollution Bulletin* 20: 304-310.

Maestrini S.Y, Berland B.R, Grzebyk D, Spano A.M (1995) *Dinophysis* spp cells concentrated from nature for experimental purposes, using size fractionation and reverse migration. *Aquatic Microbial Ecology* 9: 177-182.

Maidak B.L, Cole J.R, Lilburn T.G, Parker C.T, Jr., Saxman P.R, Stredwick J.M, Garrity G.M, Li B, Olsen G.J, Pramanik S, Schmidt T.M, Tiedje J.M (2000) The RDP (Ribosomal Database Project) continues. *Nucleic Acids Research* 28: 173-174.

Marasigan A.N, Sato S, Fukuyo Y, Kodama M. (2001) Accumulation of a high level of diarrhetic shellfish toxins in the green mussel *Perna viridis* during a bloom of *Dinophysis caudata* and *Dinophysis miles* in Sapian Bay, Panay Island, the Philippines. *Fisheries Science* 67: 994-996.

Marasovic I. (1989) Encystment and excystment of *Gonyaulax polyedra* during a red tide. *Estuarine, Coastal and Shelf Science* 28: 35-41.

Marr J.C, Jackson A.E, McLachlan J.L. (1992) Occurrence of *Prorocentrum lima*, a DSP toxin-producing species from the Atlantic coast of Canada. *Journal of Applied Phycology* 4: 17-24.

Matsuoka K, Fukuyo Y, Praseno D.P, Adnan Q (1998) *Pyrodinium bahamense* cysts in surface sediments of Jakarta bay and off Ujung Pandang, Indonesia. In: Reguera B, Blanco J, Fernandez ML, Wyatt T (eds) Harmful Algae. IOC-UNESCO, Paris, p 63.

McCook L.J. (1999) Macroalgae, nutrients and phase shifts on coral reefs: scientific issues and management consequences for the Great Barrier Reef. *Coral Reefs* 18: 357-367.

McKinnon D.A. Richardson A.J. Burford M.A, Furnas M.J. (2007) Vulnerability of Great Barrier Reef plankton to climate change. In: Johnson J.E, Marshall P.A (eds) Climate Change and the Great Barrier Reef. Great Barrier Reef Marine Park Authority and Australian Greenhouse Office, Australia, p 121-152.

Meinesz A, Belsher T, Thibaut T, Antolic B, Mustapha K.B, Boudouresque C-F, Chiaverini D, Cinelli F, Cottalorda J-M, Djellouli A, El Abed A, Orestano C, Grau A.M, Ivesa L, Jaklin A, Langar H, Massuti-Pascual E, Peirano A, Tunesi L, de Vaugelas J, Zavodnik N, Zuljevic A. (2001). The introduced green alga *Caulerpa taxifolia* continues to spread in the Mediterranean. *Biological Invasions* 3: 201-210.

Meinesz A, Devaugelas J, Hesse B, Mari X. (1993) Spread of the introduced tropical green-alga *Caulerpa taxifolia* in Northern Mediterranean waters. *Journal of Applied Phycology* 5: 141-147.

Meunier F.A, Mercado J.A, Molgo J, Tosteson T.R, deMotta G.E. (1997) Selective depolarization of the muscle membrane in frog nerve-muscle preparations by a chromatographically purified extract of the dinoflagellate *Ostreopsis lenticularis*. *British Journal of Pharmacology* 121: 1224-1230.

Morton S.L, Moeller P.D.R, Young K.A, Lanoue B. (1998) Okadaic acid production from the marine dinoflagellate *Prorocentrum belizeanum* Faust isolated from the Belizean coral reef ecosystem. *Toxicon* 36: 201-206.

Morton S.L, Norris D.R, Bomber J.W. (1992) Effect of temperature, salinity and light intensity on the growth and seasonality of toxic dinoflagellates associated with ciguatera. *Journal of Experimental Marine Biology and Ecology* 157: 79-90.

Neil K.M, Sheaves J, Cruz J.J, Hoedt F.E, Choat J.H. (2001) Port baseline surveys and surveys for introduced marine pests: the Port of Townsville, James Cook University, Townsville.

Novelli A, Kispert J, Fernandez-Sanchez T, Torreblanca A, Zitko V (1992) Domoic acid-containing toxic mussels produce neurotoxicity in neuronal cultures through a synergism between excitatory amino acids. *Brain Research* 577: 41-48.

Park M.G, Kim S, Kim H.S, Myung G, Kang Y.G, Yih W (2006) First successful culture of the marine dinoflagellate *Dinophysis acuminata*. *Aquatic Microbial Ecology* 45: 101-106.

Patil J, Gunasekera R, Deagle B, Bax N, Blackburn S (2005) Development and evaluation of a PCR based assay for detection of the toxic dinoflagellate, *Gymnodinium catenatum* (Graham) in ballast water and environmental samples. *Biological Invasions* 7: 983-994.

Pfeister L.A, Anderson D.M. (1987) Dinoflagellate reproduction. In: Taylor F.J.R. (ed) The Biology of Dinoflagellates. Blackwell, Oxford, p 611-648.

Pitcher G.C, Calder D. (2000) Harmful algal blooms of the southern Benguela Current: a review and appraisal of monitoring from 1989 to 1997. *South African Journal of Marine Science-Suid-Afrikaanse Tydskrif Vir Seewetenskap* 22: 255-271.

Quilliam M.A, Ross N.W. (1996) Analysis of diarrhetic shellfish poisoning toxins and metabolites in plankton and shellfish by ion-spray liquid chromatography-mass spectrometry. In: Snyder P.A (ed) Biochemical and Biotechnological Applications of Electrospray Ionization Mass Spectrometry, Vol Series 619. American Chemical Society Symposium, Washington DC, p 351-364.

Rhodes L.L, Mackenzie A.L, Kaspar H.F, Todd K.E (2001) Harmful algae and mariculture in New Zealand. *ICES Journal of Marine Science* 58: 398-403.

Rhodes L.L, Scholin C (2000) *Pseudo-nitzschia multistriata* in New Zealand. New Zealand *Journal of Marine and Freshwater Research* 34: 463-467.

Rizzo P.J (1981) Comparative aspects of basic chromatin proteins in dinoflagellates. *Biosystems* 14: 433-443.

Rizzo P.J, Jones M, Ray S.M. (1982) Isolation and properties of isolated-nuclei from the Florida red tide dinoflagellate *Gymnodinium breve* (Davis). *Journal of Protozoology* 29: 217-222.

Sako Y, Hosoi-Tanabe S, Uchida A. (2004) Fluorescence in situ hybridization using rRNA-targeted probes for simple and rapid identification of the toxic dinoflagellates *Alexandrium tamarense* and *Alexandrium catenella*. *Journal of Phycology* 40: 598-605.

Scholin C.A, Anderson D.M. (1994) Identification of group- and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae) I. RFLP analysis of SSU rRNA genes I. *Journal of Phycology* 30: 744-754.

Scholin C.A, Herzog M, Sogin M, Anderson D.M. (1994) Identification of group- and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). II. sequence analysis of a fragment of the LSU rRNA gene. *Journal of Phycology* 30: 999-1011.

Seliger H.H, Carpenter J.H, Loftus M, McElroy W.D. (1970) Mechanisms for the accumulation of high concentations of dinoflagellates in a bioluminescent bay. *Limnology and Oceanography* 15: 234-245.

Shulman MJ, Robertson D.R. (1996) Changes in the coral reefs of San Blas, Caribbean Panama: 1983 to 1990. *Coral Reefs* 15: 231-236.

Spector D.L. (1984) Dinoflagellate Nuclei. In: Spector DL (ed) Dinoflagellates. Academic Press, Orlando, Florida, p 107-148.

Steidinger K.A. (1993) Some taxonomic and biologic aspects of toxic dinoflagellates. In: Falconer IR (ed) Algal Toxins in Seafood and Drinking Water. Academic Press, London, p 1-28.

Subba Rao D.V, Pan Y, Zitko V, Bugden G, Mackeigan K. (1993) Diarrhetic shellfish poisoning (DSP) associated with a subsurface bloom of *Dinophysis norvegica* in Bedford Basin, Eastern Canada. *Marine Ecology-Progress Series* 97: 117-126.

Tester P, Steidinger K.A. (1997) *Gymnodinium breve* red tide blooms: initiation, transport and consequences of surface circulation. *Limnology and Oceanography* 45: 1039-1051.

Tester P.A, Stumpf R.P, Vukovich F.M, Fowler P.K, Turner J.T. (1991) An expatriate red tide bloom: transport, distribution and persistence. *Limnology and Oceanography* 36: 1053-1061.

Tindall D.R, Miller D.M, Tindall P.M. (1990) Toxicity of *Ostreopsis lenticularis* from the British and United States Virgin Islands. In: Graneli E, Sundstrom B, Edler L, Anderson DM (eds) Toxic Marine Phytoplankton. Elsevier, New York, p 424-429.

Tosteson T.R, Ballantine D.L, Tosteson C.G, Hensley V, Bardales A.T. (1989) Associated bacterial flora, growth, and toxicity of cultured benthic dinoflagellates *Ostreopsis lenticularis* and *Gambierdiscus toxicus*. *Applied Environmental Microbiology* 55: 137-141.

Tyrrell J.V, Bergquist P.R, Saul D.J, MacKenzie L, Bergquist P.L. (1997) Oligonucleotide probe technology as applied to the study of harmful algal blooms. *New Zealand Journal of Marine and Freshwater Research* 31: 551-560.

Tyrrell J.V, Connell L.B, Scholin C.A. (2002) Monitoring for *Heterosigma akashiwo* using a sandwich hybridization assay. *Harmful Algae* 1: 205-214.

Usup G, Pin L.C, Ahmad A, Teen L.P. (2002) Phylogenetic relationship of *Alexandrium tamiyavanichii* (Dinophyceae) to other *Alexandrium* species based on ribosomal RNA gene sequences. *Harmful Algae* 1: 59-68.

Van Dolah F.M. (2000) Marine algal toxins: Origins, health effects, and their increased occurrence. *Environmental Health Perspectives* 108: 133-141.

Villac M.C, Roelke D.L, Chavez F.P, Cifuentes L.A, Fryxell G.A. (1993) *Pseudonitzschia australis* and related species from the west coast of the U.S.A.: occurrence and domoic acid production. *Journal of Shellfish Research* 12: 457-465.

Villareal T.A, Roelke D.L, Fryxell G.A. (1993) Occurrence of the toxic diatom *Nitzschia pungens* f. *multiseries* in Massachusetts Bay, Massachusetts, USA. *Marine Environmental Research* 37: 417-423.

Yasumoto T, Murata M. (1993) Marine Toxins. *Chemical Reviews* 93: 1897-1909.

Yasumoto T, Oshima Y, Sugawara W, Fukuyo Y, Oguri H, Igarashi T, Fujita N. (1980) Identification of *Dinophysis fortii* as the causative organism of diarrhetic shellfish poisoning. *Bulletin of the Japanese Society of Scientific Fisheries* 46: 1405-1411.

Yasumoto T, Seino N, Murakami Y, Murata M. (1987) Toxins produced by benthic dinoflagellates. *Biological Bulletin* 172: 128-131.

Zhou Z.H, Miwa M, Hogetsu T. (1999) Analysis of genetic structure of a *Suillus grevillei* population in a *Larix kaempferi* stand by polymorphism of inter-simple sequence repeat (ISSR). *New Phytologist* 144: 55-63.

Further information

Marine and Tropical Sciences Research Facility
PO Box 1762
CAIRNS QLD 4870

or

Marine and Tropical Sciences Research Facility
PO Box 772
TOWNSVILLE QLD 4810

This document is available for download at http://www.rrrc.org.au/publications

Credits: **Southern cassowary** Wet Tropics Management Authority; **Hill Inlet in the Whitsundays** Department of Foreign Affairs and Trade - Overseas Information Branch; **Butterfly fish** Robert Thorn; **Rainforest fruits** Wet Tropics Management Authority; **Satellite image** compiled by Environmental Resources Information Network (Department of the Environment, Water, Heritage and The Arts); **Wet Tropics coast** Kerry M. Neil; **Rainforest** C. Totterdell and the Australian Heritage Photo Library.