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Chapter: **Harmful algae and their commercial implications**

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1. Introduction

Micro-algae are an important source of primary production in the oceans' food webs and are therefore beneficial to humankind. Of the approximately 5000 living micro-algae species known, 6% multiply to form harmful algae blooms (HAB). Less than 2% of the described species produce potent biotoxins (Hallegraeff 2014), which may be directly toxic to shellfish or fish, or toxic to animals or humans consuming contaminated seafood.

The key economic impacts of HABs are: (i) public health, (ii) commercial fisheries, (iii) recreational and tourism and (iv) monitoring and management costs. In an effort to assess the impact of HABs in U.S. waters, and to link research with policy, a "National assessment of harmful algal blooms in U.S. waters" has been published (CENR 2000). At that time, the average total impact was calculated as between US$34M and $82M over the six years data interrogated, with public health and commercial fisheries having had the greatest economic impacts. The cost of monitoring programmes, while giving protection to the industries, is in itself expensive, with its own economic burden for the industries (Anderson et al. 2000).

In the case of shellfish aquaculture, if shellfish become contaminated with biotoxins, and harvesting is delayed until toxin depuration is confirmed, there is the potential risk of shellfish spawning and losing condition. For the finfish aquaculture industry, fish cages may be towed to non-HAB areas and the stock saved. In the wild, fish will generally escape HAB impacts, unless trapped in bays or harbours. In the latter case, deoxygenation of the water or clogging of gills by HABs may cause death rather than biotoxins (Jones and Rhodes 1994).

Direct human illnesses associated with HABs do occur, for example, respiratory illness from breathing aerosolised micro-algal particles, but the costs to public health services from such events, and from lost work hours, are unknown (Backer et al. 2003).

As the scale of production increases, bioprocess engineering challenges and safety issues will also increase (García Camacho et al. 2007). Advances continue in methods of detection and enumeration of HABs and new molecular tools (Rhodes et al. 2013; Wood et al. 2013) and chemical tests (McNabb 2014) support public health and marine farm and biotechnology industry management strategies. In fact, HAB toxins may bring economic rewards when mass produced and processed, for use, for example, as standards for chemical testing. Patents and patent applications are now starting to appear in the literature regarding dinoflagellate toxins, although mostly for the production and extraction/purification of toxins rather than for new products for medicine or industry (Gallardo-Rodríguez et al. 2012).

2. Public health: direct impacts of HABs on micro-algae products

Many and varied products are produced directly from micro-algae, f such as aquaculture and animal feeds, renewable fuels, pharmaceuticals and health supplements. Micro-algae feed stocks were worth US$700M one decade ago (Pulz and Gross 2004) and production continues to increase globally. The health food market is valued at many millions of dollars and includes production of carotenoids, polyunsaturated oils and antioxidants to name a few.

Many thousands of metric tons of *Arthrospira platensis* (previously known as *Spirulina platensis* and commonly marketed as 'spirulina') are produced every year. Spirulina is produced in at least 23 countries, with key producers being the USA, China, Thailand and India (Takenaka and Yamaguchi 2014). There are reports suggesting producers can earn approximately US$15M per year from an annual production of 1000 tons. The main use is as a dietary supplement for human consumption, but it is also used as a supplement in animal feeds, in particular for farmed fish and poultry.

Public concern regarding cyanobacteria and their toxins has increased in recent years, due to an increase in the prevalence of blooms and a greater awareness of the health risks associated with toxic species. Cyanotoxins can cause various health issues from gastrointestinal disturbances to liver cancer and neurological impairment. Spirulina itself is non-toxic and safe for consumption, but, because photobioreactors and closed systems are costly to operate, open pond raceway systems are commonly used, and contamination by toxic species is a possibility. Cyanobacterial poisonings are well documented (Woodhouse et al. 2014) but there have been no reported illnesses related to cyanotoxins in spirulina products, probably due to well managed monocultures of *A. platensis*. However, to remove the perceived risk of contamination, a Technical Booklet for the Microalgae Biomass Industry was produced as a guide to the use of assays for detection of two cyanotoxins (microcystins and nodularins) in human food supplements (Gershwin and Belay 2009).

Anatoxin-a is an unregulated cyanotoxin that has been detected in dietary supplements. In one study, 7.7% of supplements tested were positive for anatoxin-a. This included one contamination each of fish, bird and human cyanobacterial food products (Rellan et al. 2009).

A potential positive economic benefit from cyanobacterial toxins is the utilisation of their ecological role as allelochemicals, which could have uses as algaecides, herbicides or insecticides (e.g. against mosquito larvae) (Berry et al. 2008).

3. Impacts of HABs on aquaculture

Figure 1. Marine farms in the Marlborough Sounds, New Zealand. Greenshell mussels™ (*Perna canaliculus*) growing on ropes (left) and caged Chinook salmon (*Oncorhynchus tshawytscha*; right). (Images Cawthron Institute, Nelson, New Zealand.)

3.1 Shellfish

Micro-algae are also the basis of farmed fish and shellfish industries and the market is rapidly expanding as the demand for seafood increases and the wild catch declines. The global aquaculture market in 2012 was estimated at US$135B and growing (Sheela 2013). The small percentage of micro-algae species that do produce toxins can impact on the seafood industry (Figure 1). However, the impacts of harmful algal blooms (HABs) on the global economies are difficult to assess as management 'around' HAB events is often practised. Losses may then be avoided, although there may be a break in the continuity of supply with associated costs.

In New Zealand, from late 1992 to early 1993, a bloom of the dinoflagellate genus *Karenia* (predominantly *K. mikimotoi*) led to a neurotoxic shellfish poisoning (NSP) event (Jasperse 1993), catching the industry and government agencies by surprise. Before this event, New Zealand's biotoxin programme was minimal, and because of the event a three-month closure of the entire New Zealand coastline was instigated, with all shellfish industry operations suspended until the cause was determined. Subsequently, on-going monitoring programmes were established.

Ireland experienced a similar unexpected setback with the emergence of a toxin in mussels harvested from Killary Harbour in 1995 (Twiner et al. 2008; Tillmann et al. 2014). The causative organism was then unknown, but contaminated shellfish were causing human illness (Furey et al. 2010). The Irish shellfish aquaculture industry, with a value of > US$65M per annum, suffered economic losses from closed markets until the issue was resolved, and a new biotoxin management plan and monitoring regime was implemented. The toxins responsible were identified as azaspiracids (AZAs), although the organism responsible for their production was not identified until more than a decade later, when the chemical-based technology of liquid chromatography coupled with mass spectrometry (LC–MS/MS) enabled the fast and reliable detection of these toxins. The small dinoflagellate producer was classified as *Azadinium spinosum* (Tillmann et al. 2009). Many other *Azadinium* species, both toxic and non-toxic, have been identified since 2007 (Tillmann et al. 2014).

Australia's Tasmanian oyster industry provides direct employment for >300 people with approximately 4 million dozen oysters harvested annually at an estimated value to the farmer of >US$20M. The mussel industry continues to expand in Tasmania with production during 2010 – 2011 of 717 tonnes, valued at US$2.3M. In late October 2012, Japanese import authorities identified paralytic shellfish toxins (PSTs) at levels above the regulatory limit in a shipment of fresh mussels (*Mytilus galloprovincialis*) from the east coast of Tasmania. Recalls from export markets were instigated for >100 tonnes of mussels harvested between September and October. The cause of the biotoxin contamination of the mussels proved to be an undetected bloom of the PST-producing dinoflagellate *Alexandrium tamarense*. The bloom was later shown to have impacted several other commercial seafood species including oysters, rock lobsters, clams and scallops (Campbell et al. 2013). Oyster producers reported that, due to the harvesting closures which ensued, about 540,000 dozen oysters valued at US$3.4M were withheld from the Australian markets. Losses flowed on to service providers. For example, freight operators had estimated losses of US$583,000. On a direct economic basis, the cost of the bloom event was estimated at US$8M, representing impacts on revenue and expenditure across all stakeholders. Since then, monitoring procedures have been reviewed and exports are again being accepted into overseas markets (Campbell et al. 2013).

The Japanese pearl oyster industry was hard hit in the 1990s by another dinoflagellate, *Heterocapsa circularisquama*, which causes bivalve mortalities. The industry has lost millions of dollars since the first event in 1998 (GEOHAB 2010).

3.2 Finfish

Asia, particularly China, has the highest production of aquaculture fish and shellfish globally (89% of global marine aquaculture production in 2006), and this is at a time when HABs are reported to be increasing (GEOHAB 2010). Causes of fish mortalities may be from hypoxia or anoxia, or from mucilage production by some micro-algae species which can clog fish gills. A key species of concern is the ichthyotoxic dinoflagellate, *Cochlodinium polykrikoides*, which caused losses of US$0.8M in western Japan in 1979 (GEOHAB 2010). Since that first report there have been even greater losses, reaching US$330M in Yatsushiro Sea in 2000. In Korea, *C. polykrikoides* caused economic losses of US$95.5M, which were borne by fish farmers, in 1995. Losses have continued over the last two decades and intensive efforts have gone into the early prediction and warning of blooms (Kim 2012). Korea now has a national monitoring programme for both shellfish and finfish HAB risks with the aim of early detection; monitoring even includes aerial helicopter surveys. Mitigation is a research focus at national research institutes and universities and a novel mitigation method has been developed which uses the settling of yellow clay particles to drag blooms down to the sediments while also providing an adsorbant surface for biotoxins (Figure 2; Kim 2012; Choi and Lee 2012).

Figure 2. (Left) Clay application to remove *Cochlodinium polykrikoides* blooms and their toxins from fish farms in South Korea (image L Rhodes); (Right) *Cochlodinium polykrikoides* isolated from South Korean coastal waters (Bar = 10 µm; image copyright Zhun LI, Hyeon HS, Myung-Soo H).

Sea-cage Chinook salmon (*Oncorhynchus tshawytscha*) mortalities have caused serious financial losses for the New Zealand finfish aquaculture industry (Figure 1) with two major events occurring in Big Glory Bay, Stewart Island, in 1989, and in the Marlborough Sounds in 2010. The causative organisms were the raphidophyte *Heterosigma akashiwo* and the dictyophyte *Pseudochattonella verruculosa* (Figure 3) respectively (MacKenzie 1991; MacKenzie et al. 2011). The salmon deaths in 1989 (>600 tonnes) were estimated to cost the industry approximately US$15M (Chang et al. 1990) and the 2010 event (losses of 200 tonnes) was only tempered by the removal of the sea cages to an area free of the bloom. *P. verruculosa* blooms have also been responsible for losses of Atlantic salmon (*Salmo salar*) in Scandinavian coastal waters (1100 tonnes in 2001; Riisberg and Edvardsen 2008) and has caused losses of a variety of fish species in Japan (Baba et al. 1995, Yamamoto and Tanaka 1990).

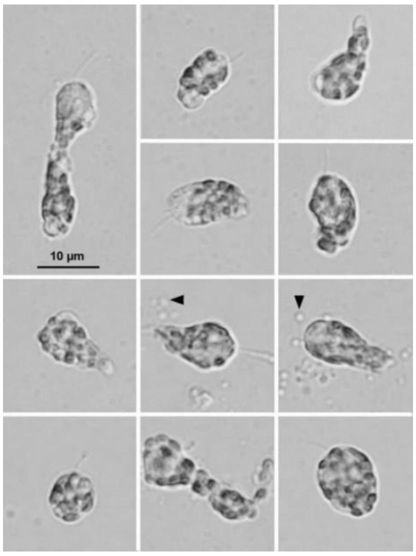

Figure 3. Light micrographs of *Pseudochattonella veruculosa*. (Image L MacKenzie, Cawthron Institute, NZ.)

In the northern summer of 1997, fish kills associated with the heterotrophic dinoflagellate, *Pfiesteria piscicida*, occurred in Maryland; complaints of health effects were also reported by local fishers. The impact was limited, but the economic impacts were huge. Consumers panicked and sales of seafood went down with a calculated loss of US$43M. The 'halo effect' also led to the avoidance of Maryland for boat charters with the recreational fishing industry also losing several million dollars. An overall economic loss of US$50M was estimated for just a four-month period (CENR 2000).

Ciguatera fish poisoning (CFP) is considered a neglected disease globally but its incidence appears to be increasing (Chinain et al. 2010). In the Pacific, no Polynesian archipelago is considered safe from CFP, with a >6-fold increase in incidences over a two decade period in the Australes, French Polynesia, alone (Chateau-Degat et al. 2007, Chinain et al. 2010). CFP results mainly from the consumption of reef fish that have grazed on microalgae harbouring the causative organisms, which are cells of toxic species in the benthic/epiphytic dinoflagellate genus, *Gambierdiscus* (Litaker et al. 2009, 2010). *Gambierdiscus*, and the associated CFP incidences, is expanding its range, even into temperate regions (Kohli et al. 2014). CFP is common in many Pacific Islands, with the greatest incidence being reported from the Cook Islands (Rongo and Woesik 2012). According to Fleming et al. (1998) the estimated global number of poisonings was 50,000 – 500,000 per year nearly two decades ago. A later report estimated 5-600 cases per 10,000 people annually, depending on the geographic region (Lange 1994). In Australia, OzFoodNet data (2001-2010) shows 283 ciguatera poisonings during that decade (Braidotti 2014). The economic impacts of CFP are largely unreported and are likely to increase in the future with the double impact of rising seawater temperatures and the acclimatisation of the dinoflagellates to cooler waters.

Lyngbya majuscula, a benthic filamentous marine cyanobacterium which occurs throughout the tropics and subtropics to depths of 30 metres, also impacts on commercial fisheries. The fish will actively avoid bloom areas but local fish stocks are affected when toxins and/or low oxygen levels lead to mortalities of fish and other aquatic organisms. Freshwater fisheries can also be impacted by cyanobacteria HABs. For example, there was an estimated loss of approximately US$200K per annum for commercial fisheries in the UK due to damages induced by cyanobacterial blooms (Pretty et al. 2003; Hamilton et al. 2014).

4. Impacts of coastal HABs on tourism

Karenia brevis has been cited as the cause of respiratory distress in beach-goers in Florida with a subsequent loss of tourism dollars, and has been responsible for the deaths of iconic sea mammals such as the manatee. The actual losses to the region are difficult to determine, but have been estimated as US420M annually (CENR 2000). The average losses by beachfront restaurants in Southwest Florida were statistically determined over a 7 year period as 13.7% - 15.3% when 'red tides' were present (Morgan et al. 2009).

Blooms of the benthic/epiphytic genus *Ostreopsis* occur throughout the Mediterranean and have been of concern since human illnesses occurred due to inhalation by beach-goers of the aerosolised toxins (palytoxin-like compounds) produced by the dinoflagellate. The bloom caused skin irritation and respiratory distress resulting in many hospitalisations. These events have the potential for similar economic impacts on tourism as have occurred in Florida. The aerosol route is the main cause of concern in this regard, although invertebrate taxa may accumulate low concentrations of the toxin with a risk of illness from consumption of these animals (Ciminiello et al. 2006, 2008; Munday 2008; Shears and Ross 2009; Rhodes 2011; Rhodes et al. 2002, 2008a, 2b). It is noteworthy that human deaths have been linked to the eating of palytoxin-contaminated crabs in the Philippines (Gonzales and Alcala 1977; Yasumoto et al. 1986).

CFP does not appear to impact on tourism at present, although many tourists, having eaten contaminated reef fish in tropical regions, show symptoms of CFP after returning to their country of origin. The symptoms range from relatively minor (diarrhoea) to serious and long-lasting neurological symptoms and even death. If such problems become more widely known, an impact on tourism in the Pacific is to be expected.

Cyanobacterial blooms are a common occurrence globally. In Australia, the toxins produced by *Lyngbya majuscula* can cause skin, eye and respiratory irritation (Osborne et al. 2001). These blooms have increased in Moreton Bay, Queensland, in recent years and the Moreton Bay Regional Council has developed a 'Lyngbya Management Strategy' and a response plan to reduce impacts of the blooms on human health and the economy. The South East Queensland's recreational fisheries have been valued at more approximately US$180K and the blooms, which occur throughout the state, can lead to a decrease in visitor numbers and thus to a significant economic impact on the tourism industry. Shore clean-ups by the former Caboolture Shire Council to remove *Lyngbya* washed onto its local beaches reached approximately US$700K over four summers.

Also in Australia, the Tasmanian PST event of 2012 had an impact on the tourist industry in that state, with impacts on accommodation and restaurants/cafes ranging from US$14K - $90K per month (Campbell et al. 2013).

5. Costs of managing HABs

The initial cost of monitoring programmes to the industry and New Zealand government was approximately US$3.6 M per annum, although this figure has since been scaled back substantially (Rhodes et al. 2013). More cost-effective biotoxin testing (e.g. the replacement of the mouse bioassay by chemical analyses) and greater reliance on phytoplankton testing and calculated biotoxin risk levels have allowed this cost saving trend to continue (McNabb et al. 2005; McNabb 2008, 2014). The success of the monitoring programmes is exemplified by the excellent management of blooms of the paralytic shellfish toxin (PST) producing HAB species *Gymnodinium catenatum*, *Alexandrium minutum* and *A. catenella* in recent years (Figure 4: MacKenzie 2014).

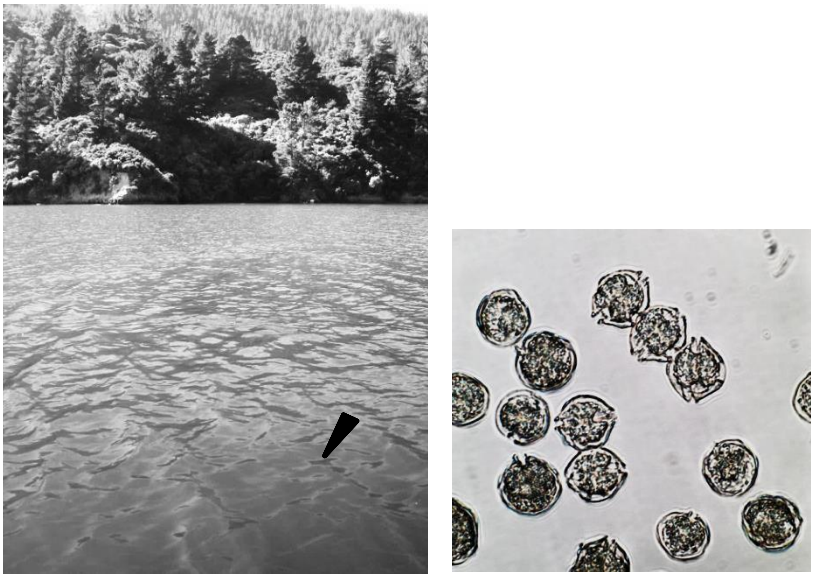

Figure 4. (Left, arrowed) A bloom of *Alexandrium catenella* in the Marlborough Sounds, New Zealand and (right) light micrographs of motile cells of *A. catenella*. (Images L MacKenzie, Cawthron Institute, NZ.)

The application of LC-MS has allowed better advice on food safety and in many cases has enabled commercial enterprises to continue to operate. Where the LC-MS has not been so readily accepted as a replacement, problems have arisen over the validity of the mouse bioassay. For example, in Arcachon, France, closures to oyster harvesting were instigated based on mouse bioassay results between 2006 and 2009 although no known micro-algal toxin was found (McNabb 2014). Similarly, closures to oyster harvesting occurred in northern New Zealand and South Australia in 2009 due to positive mouse biosassays; the toxins were later shown to be the unregulated pinnatoxins (Rhodes et al. 2010, 2011).

Because spirulina is considered a dietary supplement in the U.S., there is no active, industry-wide regulation of its production and no enforced safety standards for its production or purity. The U.S. National Institute of Health describes spirulina supplements as "possibly safe", provided they are free of microcystin contamination, but "likely unsafe" (especially for children) if contaminated. The fear of toxic contaminants entering cultured blue-green algae (BGA) health supplements, and the detection of microcystins at low levels in some products (Heussner et al. 2012) has led to the setting of regulations by the Oregon Health Division and the Oregon Department of Agriculture. They established a regulatory limit of 1 µg/g for microcystins in BGA-containing products (Gilroy et al. 2000). Many producers choose to attain certification (e.g., USDA NOP, Ecocert, OCIA–IFOAM) and if the producer is approved (e.g., USP, HACCP, BVQI ISO 9001, ISO 14001) the risk of toxins is virtually eliminated.

In conclusion, the impact of HABs can be devastating, particularly for those countries that have a high dependence on seafood. As coastal waters continue to be overfished, aquaculture is seen as the obvious alternative. It is expected that the value of world aquaculture production will soon out-perform the economic returns from the total catch of wild fish and shellfish (Hallegraeff 2003). As more of the coastal 'space' is given over to marine farms it is likely HABs that were of no consequence previously will begin to pose threats. The implementation of monitoring programmes and the advent of faster, cheaper test methods are reducing marine farm closures and minimising product recall from markets, and this will be critical for a world dependent on seafood through aquaculture.

A continuing trend is the development of valuable products from algae. Commercial developments based on micro-algae require certified materials and in this regard culture collections are critical to ensure that strains are kept pure. Where possible, cryopreservation in curated collections also ensures physiological processes (e.g. toxin production) remain unaltered (Wood et al. 2008).

Changes in the climate globally and the increasing acidification of our oceans are likely to impose unexpected impacts with unforeseen economic stresses and these impacts can be expected on pond production as well as coastal aquaculture. Prediction is a challenge but will be even more critical in the future as global populations grow and reliance on farmed seafood and products from cultured micro-algae increases.

Figure 5. Semi-continuous bag cultures of micro-algae for production of valuable compounds (image G Stirling).

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