Chapter 1

Dynamics of phytoplankton in relation to tuna fish farms in Boston Bay, and near-shore Spencer Gulf, South Australia

Introduction

Phytoplankton are unicellular algae, which are the base of the food web in the oceans. They come in many forms and sizes such as diatoms, dinoflagellates and nanoflagellates. Amongst the many thousands of species of phytoplankton approximately 40 species are known to be harmful to humans and marine organisms (Hallegraeff 1995; Silke *et al.* 2005). Harmful species include: *Chattonella marina*, *Karenia brevis*, *K. mikimotoi*, *Prorocentrum micans*, *Alexandrium minutum*, *Dinophysis acuminata*, *D. acuta*, and *Ceratiurm fusus.* The toxic species in bloom are known as harmful algal blooms or HABs. HAB species can occur in geographically separated and environmentally distinct regions, and as a result, may differ in their growth dynamics and expression of toxin production (Anderson *et al*. 2005).

Blooms of phytoplankton may occur due to a complex of environmental parameters including turbulence, winds, currents, temperature, salinity, nutrient input, land-run-off and precipitation. They are natural phenomena, which have occurred throughout recorded history (Hallegraeff 1995; Silke *et al.* 2005); however, in recent decades such events have increased in frequency, intensity and geographic distribution (Anderson *et al.* 2005). HABs are responsible for severe economic damage to the aquaculture

industry worldwide. For example, mortality of farmed fish species yellowtail (*Seriola quinqueradiata*) and other fish species during HAB outbreaks in the Seto Inland Sea of Japan between 1972 and 1991 cost farmers in excess of $161 million (Honjo 1994; Fukuyo *et al.* 2002). Interest in the effect of fish farming practices on the marine environment has arisen because there is concern that the wastes that fish farms produce may be contributing to the problem of HABs.

Fish farming in South Australia

Fish farming in Australia is an expanding industry, the largest of which are salmon farming in Tasmania and tuna feed lots in Boston Bay, South Australia (Figure 1). Tuna fish farming began in Boston Bay in 1991 after the collapse of the southern bluefin tuna (*Thunnus maccoyii*) wild fishing industry. Tuna fish farmers are reliant on the capture of wild southern bluefin tuna (*Thunnus maccoyii*) under a quota system directed by the Australian Fisheries Management Authority (AFMA). Wild tuna are captured along the continental shelf during their annual migration and brought into Boston Bay during the months November to January. The fish are transferred to cages 50 m in diameter and fed pilchards to increase their weight before marketing to Japan in the later part of the year. Tuna farming is the largest single sector of the State's aquaculture industry. Tuna fish farms are situated in and just outside Boston Bay, Spencer Gulf and Louth Bay in South Australia.

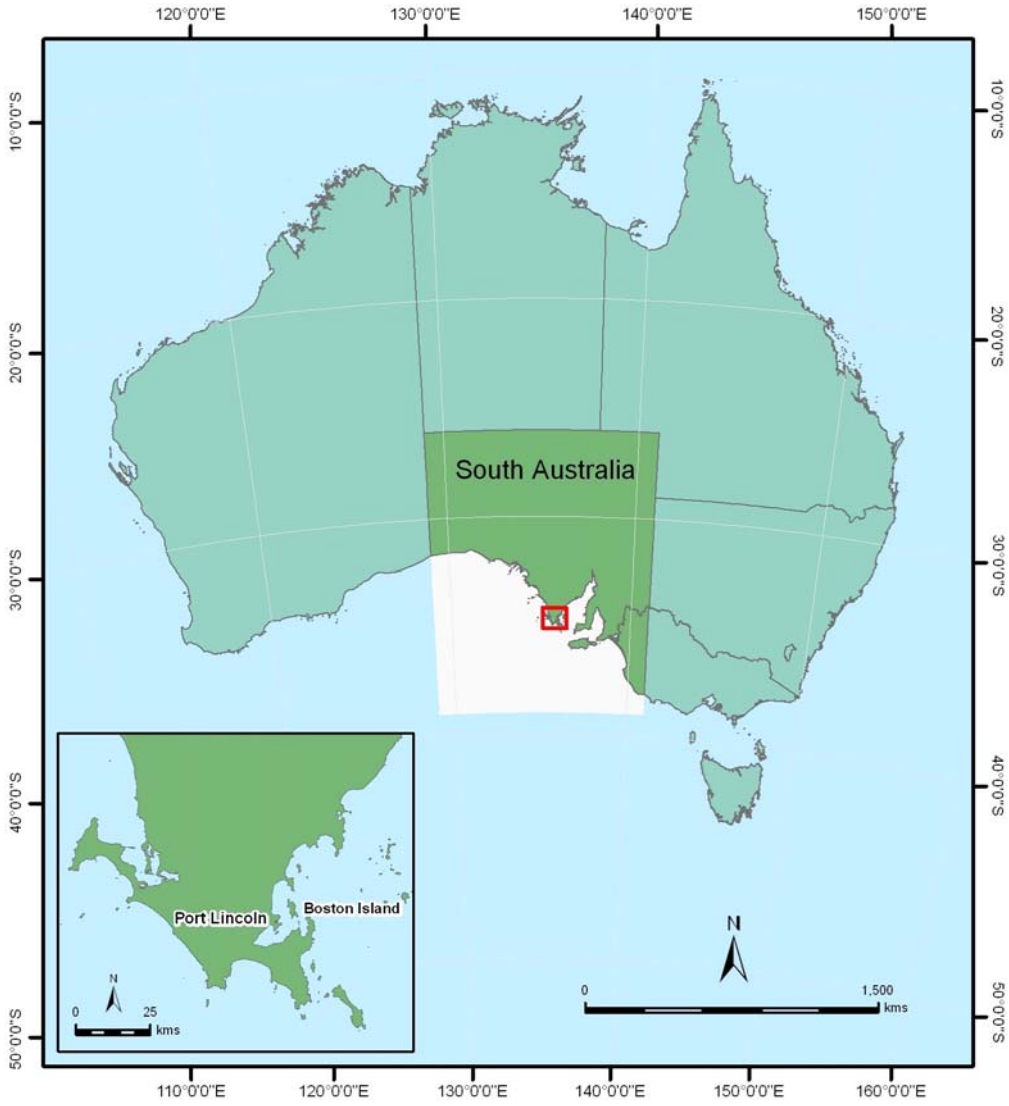


Figure 1: Map showing Boston Bay, Port Lincoln, South Australia.

Boston Bay is a north-south aligned bay situated on the south-eastern side of Eyre Peninsula, South Australia. It is approximately 17 km long and 6 km wide with a maximum depth of about 20 m. Boston Island, which is 7 km long and 3 km wide, is located in the middle of the bay. Channels at the northern and southern ends of the island allow exchange between Boston Bay and Spencer Gulf (Bond 1993). Boston Bay is linked to Proper Bay and Spalding Cove. Winds for most of the year are gentle breezes (<10 k). Gale force winds are uncommon (1%) and occur mostly in spring.

Data suggests that currents are strongly influenced by local wind and atmospheric effects. Current speed is much slower inside the bay (<2.5 cm/s 75% of the time), than in the southern channels (<12.2 cm/s 75% of the time) (Petrusevics 1993).

Very large currents can be seen sweeping past the east of Boston Island, up the Gulf, in contrast to small currents observed inside Boston Bay and Proper Bay. When the tide turns in Boston Bay, the water in Boston Bay is virtually stationary and currents in Boston Bay and Proper Bay are almost negligible. However, currents still remain large in Spencer Gulf. This suggests that pollutants or organisms located on the western side of Boston Island will tend to accumulate in Boston Bay (Noye 1996). Tides rise and fall by approximately 1 m in Boston Bay, over a 6–7 hour period, depending on the time of the year and phase of the moon. Neap tides, which result in negligible tidal movement for periods of up to 24 hours, occur each fortnight, and are commonly known as dodge tides (Engineering and Water Supply Department 1976). Flushing time in Boston Bay is 9 days in winter and 24 days in summer (Petrusevics 1993). Boston Bay waters are well mixed with salinities of approximately 35 and temperatures range between 12^{o}C in the winter to 23^{o}C in the summer (Paxinos *et al.* 1996).

Anthropogenic inputs, other than tuna farms, into the Bay come from: primary treated effluent from the Billy Lights Point Engineering and Water Supply Department sewage treatment plant; small creeks which run into the bay; small industries including an oil terminal; fish factories; and an

abattoir which also discharges waste. Shipping channels are also dredged and there is much activity from the loading of grain and fertiliser from which the dust enters the marine environment.

Studies in Boston Bay and Spencer Gulf have been conducted since the early 1970s mainly on the benthic environment and water quality (Deland and Jones 1973; Engineering and Water Supply Department 1976; Shepherd 1975; Department of Agriculture and Fisheries 1975; Caldwell Connell Engineers 1976; Walters 1989; Harbison and Wiltshire 1993). These studies investigated sewage and fish factory discharges into Proper Bay and the effects on water quality and marine benthic organisms. The volume of sewage discharged into this area is 945 megalitres/yr containing approximately 10.4 t of nitrogen and 7.1 t of phosphorous. There were was some concern that this pollution would create sinks for nutrients or other pollutants in the shallow bays of Spencer Gulf (Engineering and Water Supply Department 1976). As intensive as some of these studies were there was no investigation into the effect of point-source pollution on phytoplankton dynamics. However, there is some background information on chlorophyll *a* and nutrient levels from a study conducted by Caldwell Connell Engineers in 1976 on sewage discharges in Proper Bay and Boston Bay.

Recorded measurements of chlorophyll *a* (a measure of phytoplankton biomass), during December 1975, at the sewage outfall in Porter Bay, the head of Proper Bay and to the southeast tip of Boston Island, ranged from 0.2 to 0.05 µg/L respectively. Nutrient levels recorded at the same time

showed nitrates and nitrites ranged from <0.01 to 0.11 mg/L in Proper Bay and Boston Bay and TKN ranged from 0.14 to 0.99 mg/L. A later study (Walters 1989) found similar nitrogen and TKN levels around the outfalls and in Boston Bay, although ammonia levels were generally high near the outfalls 1.5 mg/L. No chlorophyll *a* measurements were taken in this later study.

However, effects on the benthos were recorded. Caldwell Connell (1976) reported that there had been a 40 ha loss of the seagrass meadows (*Posidonia australis*) in Proper Bay: the growth of *Posidonia* was stunted with dense layers of epiphytes and black spots recorded on the fronds. There was also evidence of an increased population of suspension and deposit feeders within 10–15 m of the sewage discharge pipe and very dense beds of the green alga *Ulva*. Steffensen (1989) later reported that in 1980 the domestic sewage outfall had been extended from 180 m to 500 m offshore increasing the depth from 2 to 16 m. Surveys around the outfall showed that the accumulation of grit and other debris had eliminated most of the biota within 10 to 15 m of the outfall; an increase in the abundance of filter feeding and detrital feeding animals; and areas previously colonised by *Posidonia australis* were either bare sand or had been recolonised by *Heterozostera.*

Studies around tuna farms in South Australia

Studies around the tuna farms have been conducted since the mid-1990s. These looked at the impact of the waste sediment on the benthic environment below the fish cages (Cheshire *et al.* 1996), dissolved oxygen

levels in and out of tuna cages (Cronin *et al.* 1999) and a study of methods to assess and manage waste dispersal and deposition from the tuna farms in Boston Bay (Paxton 1997). Other monitoring programs have also given regular nutrient and chlorophyll *a* reports from Boston Bay and Spencer Gulf (Clarke 1996, Clarke *et al.* 2000; Paxinos *et al*. 1996; SA Water unpublished data 1995 to 2000). These studies showed that background levels of chlorophyll biomass in Boston Bay are quite low during the spring months, from September to November, and largest during the autumn months from March to May. A small-scale qualitative survey was conducted on phytoplankton biomass, abundance and species composition around tuna fish farms in Boston Bay during March/June and September/December of 1995 (Paxinos *et al.* 1996). The potentially toxic phytoplankton found were the diatoms *Pseudonitzschia* sp. and *Chaetoceros* spp., the dinoflagellates *Gymnodinium sanguineum*, *Dinophysis acuta* and *D fortii*, *Gonyaulax* spp., *Scrippsiela* sp. and *Ceratium fusus*.

A fish kill occurred in Boston Bay in April 1996 and $A80 million worth of tuna died. It is still not known if this was the direct result of a bloom of the toxic phytoplankton *Chattonella marina* or the fault of an unusually large storm, which hit Port Lincoln just before the fish kill (Clarke 1996; Noye 1996; Munday and Hallegraeff 1998). Algal blooms are common in Boston Bay and Spencer Gulf with the most extensive being the blue green algae *Trichodesimium erythaeum*. *T. erythaeum,* which always blooms in January, when conditions are warm and calm, and which can stretch for a length of 20 nm from the Sir Joseph Banks Group of Islands,

north west of Boston Bay, to Taylors Island in the south. In 1997 shortly after this bloom, *Gymnodinium breve* (like) (now *Karenia brevis*) and *G. mikimotoi,* (now *K. mikimotoi*) cells were recorded in Boston Bay. These events highlighted the necessity for research and monitoring of water quality and sediments around fish farms. Currently, monitoring of the benthic environment around fish farms takes place once a year (Loo *et al.* 2006) and the EPA (Environment Protection Authority) and records water quality measurements. No long-term monthly/weekly monitoring takes place around the fish farms. The purpose of this study was to examine phytoplankton species composition, distribution and abundance in Boston Bay, and Spencer Gulf in relation to tuna fish farm activities. At the time of this study there was little information on phytoplankton in Boston Bay and Spencer Gulf.

The following section gives the definitions of important terms used in this document. “Hyper-nutrification” is defined as any substantial and measurable increase in the concentration of dissolved nutrients (Gowen and Bradbury 1987). The term “eutrophication” is commonly used in relation to areas that have suffered hyper-nutrification and thereby become highly productive in terms of organic matter formed (Gowen and Bradbury 1987). Nixon (1995) defined eutrophication similarly as the increase in the rate of supply of organic matter to an ecosystem, emphasising the idea that eutrophication is a process and not a trophic state.

How is eutrophication measured and what are the acceptable limits? There are few standards, but for Northern European waters a limit of 10 µg/L for chlorophyll *a* has been recommended as an environmental water quality standard (CSTT 1994). In most cases it appears that determining eutrophication levels is arbitrary. They are determined by the level of chlorophyll *a*, sustained over a particular time period (sometimes only two or three days), that is over and above that of prior recorded natural variation. Australian water quality measurements including measures of eutrophication are based on the water quality requirements that support the designated environmental values in the Australian Guidelines for Fresh and Marine Waters (ANZECC 2000). For South Australia the acceptable levels for chlorophyll *a,* according to ANZECC guidelines, is 1 µg/L (ANZECC 2000). The following review examines fish farm practices globally and their effects on the local marine environment in terms of sediment and water quality and how this may affect phytoplankton dynamics.

Fish farming and waste produced

Farmed fish produce solid waste from uneaten food and faeces. This waste falls to the seabed and then forms sediment under the cages (Pearson and Rosenberg 1978; Weston and Gowen 1988; Caine 1989; Gowen and Rosenthal 1993; Findlay *et al.* 1995; Tsutsumi 1995; Cheshire *et al.* 1996; Herman 1997; Troell and Berg 1997). Soluble waste is created directly through the production of urinary ammonia and urea excreted by farmed fish and results in the formation of nitrogen or ammonia that is directly input into the water column (Enell and Lof 1983; Enell and

Ackefors 1992; Edwards 2001; Bongiorni *et al.* 2003). The elevated nutrient at fish farm sites is a potential stimulant of intense local blooms that can provide seed populations delivered through advective spreading leading to blooms elsewhere (Smayda 2006).

The significance of nutrient wastes discharged at fish farms becomes more evident when compared to sewage treatment loadings (Smayda 2006). For example, 8,700 t of the 10,000 t of salmon produced in Scotland in 1986 were farmed on the West and North coasts and the Hebridean Islands. The amount of nutrients discharged from fish farms in that region that year was estimated to have exceeded that in the waste from the human population in that region (Tett and Edwards 2002). In Canada the amount of waste input from fish farm sites equaled the untreated municipal sewage of a city of 300,000 (Smith *et al.* 2001). Holby and Hall (1991) calculated that the phosphorus excreted from a fish farm of rainbow trout (*Onchorhynchs mykiss*) in Sweden with an annual production on only 50 t would be equal to the sewage discharge from a town of 7,000 people.

Nitrogen may also become available to the water column from fish farming activities, through remineralisation of uneaten food and resuspension from sediments. In the case of remineralisation, studies have shown that the amount of nitrogen that is available from remineralisation of uneaten food during ‘sinking’ is minimal (Gowen and Bradbury 1987). This suggests that the major part of additional nitrogen to the water column is derived from soluble nitrogen (N) as urinary ammonia

and urea, excreted by farmed fish and nutrients resuspended from the sediments.

There is some evidence for resuspension of nutrients from sediments. For example, Kaspar *et al.* (1988) have shown that nutrients are not resuspended from sediments directly beneath the fish cage but at a distance from them. The feed required to produce 1 t of fish contains 110–130 kg N. Of this N, 20–25% is retained in the fish and the remainder is either not ingested or converted to waste products (ammonium and dissolved organic N, 55–65%; sedimenting solids, 35–45%). The products of sediment biodegradation (dissolved organic N, ammonium) diffuse back into the water column so that eventually more than 90% of the waste N (90–100 kg N per t fish) is dissolved in the water column and less than 10% of the waste N is buried in the sediment. To determine how much nitrogen was locked into the waste sediment, Kaspar *et al.* (1988) conducted a study that determined the nitrification and nitrate reduction rates in sediments in the vicinity of a sea cage salmon farm in New Zealand.

The study found that the vertical distribution on sediment microbial characteristics resulting from salmon farm waste sediment was well illustrated by the denitrification activity. For example, at site 1, under the centre of the cage, there was no activity to a depth of 6 cm. At site 2, 4 m from the cage edge, showed a maximum between 2 and 6 cm and at the furthermost sites, 10 and 30 m from the cage, the highest activities were in the surface layer. It was expected that these changes would spread over larger areas and greater sediment depths as the waste input continued.

Kaspar *et al.* (1988) concluded that denitrification in the vicinity of the salmon farm was not a significant N removal mechanism. Therefore, it was assumed that more than 90% of the waste N (90–100 kg N per t fish produced) was made available in the wider environment of the salmon farm for phytoplankton use. Essentially, this is suggesting that N was not available directly under the farm cage but around the cage area to 30 m and in a form that would promote phytoplankton growth.

Depending on the hydrography of the site of the farm there may be differences in the way that resuspension of nutrients from sediment takes place. A study of trout fish farm sediment from net pen cages in Kolding Fjord,Denmark (Holmer and Kristensen 1992) showed that metabolism is an order of magnitude higher in sediment underlying net cages, compared to a control site 30 m away and to rates measured in unaffected coastal areas. This has been found in previous studies on sediment from fish farms (Holby and Hall 1991). Anaerobic microbial processes degraded most organic matter and recycling of nutrients was quite rapid in the sediment. It was concluded that the release of nutrients contributed to hyper-nutrification around the fish farm and that as much as 80% of N and 60% of P wastes ended up in the water column.

Eutrophication was not measured in this study and there was little mention of the hydrography and oceanography (such as current rates) of the two study sites, so it is difficult to assess why differences between the two sites were found. In both studies it is evident that there is some resuspension of N from sediments under fish cages which suggests that

the main contributor to soluble nutrients from fish farm practices is through the production of soluble N.

The production of soluble N can be estimated by looking at fish farm waste loadings; however, these estimates may alter at different temperature regimes. A salmon farm in temperate waters with an annual production of 200 t of fish produces an annual loading of 2 t of phosphorus (P), 18 t of nitrogen (N) and 100 t of oxygen (O_2) consumed through BOD (Seymour and Bergheim 1991). In tropical waters, rates measured by Wu *et al.* (1994) in Hong Kong found that 1.8 t of fouling material produced per ton fish is equivalent to 31 kg BOD, 7.5 kg N and 70 kg P. Rates also vary with the size and type of fish being produced.

For southern bluefin tuna (*Thunnus maccoyi*) farmed in Boston Bay, South Australia, it was found that they have a wet-to-wet feed conversion rate in the vicinity of 17:1 (Smart 1996). There were approximately 5,000 t of tuna farmed in Boston Bay and Spencer Gulf annually from late November to around August from 1996–2000 (Clarke *et al.* 2000) (this figure has since doubled). The tuna were fed approximately 260 t of pilchards per day, which is 9% of body weight. Using the feed conversion ratio, 15 t of tuna would produce approximately 245 t of waste per day, of which 60–70% is urinary ammonia and urea (Smart 1996). Soluble N then remaining in the local marine environment, given optimal conditions, may create hyper-nutrification and then subsequent eutrophication. This is dependent on the oceanography and hydrography of the area in question.

Contribution of oceanography and hydrography to phytoplankton and fish farm ecology

Turbulence, winds, currents, temperature, salinity, nutrient input, land-run-off and precipitation and depth of water act together in the dispersion of nutrients around a fish farm. Dispersion creates a variable distribution of phytoplankton biomass, often with areas of elevated phytoplankton concentrations referred to as patches. Thus, the physical parameters determine factors such as the position and intensity of blooms, as well as how long they last and their spatial extent.

Early studies of oceanography around a fish farm (Gowen and Bradbury 1987) showed that exchange rates of water around farms contribute to the final level of phytoplankton enrichment. However, they could only speculate that the release of dissolved forms of nitrogen from fish farms, together with ammonium released from anoxic enriched sediments, may result in hyper-nutrification and eutrophication. It was suggested that localised eutrophication might occur around large farms in poorly flushed locations but at the level of salmonid farming at the time of the study widespread eutrophication was unlikely. Therefore for a fish farm site there must be ample current flow and depth in order to safely disperse the waste feed and fish faeces produced by fish farms (Caine 1989). Furthermore, the water needs to be deep enough to avoid anoxia of bottom sediments. For example, Tsutsumi (1995) reported that organic enrichment from farms progressively produced anoxia in bottom waters and also hyper-nutrification of the water column at fish net pens in Tomoe Cove, Japan. Tsutsumi (1995) concluded that the semi-enclosed topography and

bathymetry of such a sheltered bay restricts water exchange, which traps inorganic nutrients and labile organic matter from the net pens.

Consequently, fish culture can cause significant hyper-nutrification of the water and marked enrichment of the bottom sediment in sheltered areas. However, this may be dealt with differently in different environments. For example in the tropical Lake Kariba, Zimbabwe, Troell and Berg (1997) suggest tropical fish farming can generate similar hyper-nutrification effects that are observed in temperate regions. But the results also indicate that a tropical lake system may be able to process local deposition of organic wastes better than a temperate one, suggesting that microbial decomposition may be a rapid and prominent process in tropical systems. This means that `flushing time' of the body of water where the fish farm is placed needs to be reasonably rapid to allow for advection of soluble nutrients away from the farm area. Currents, however, may be slowed around a fish farm given the solid structure of the fish farm cage. If the effect is significant, it may also indirectly contribute to hyper-nutrification (Silvert and Sowles 1996).

Thus, oceanography and hydrography at the fish farm site may not only contribute to localised hyper-nutrification but also nutrients advected away from the fish farm area can alter the water quality at a distant site. It is therefore instrumental to view the input of waste from fish farms into the marine environment as nutrient pulses, which may intermittently affect phytoplankton growth over an indefinable area.

Spatial and temporal distribution of phytoplankton around fish farms

Much of the literature discusses the possibility of localised blooms from fish farm waste; however, there are several ways by which phytoplankton could respond to nutrient-pulses produced from a fish farm site. Some of these options are discussed below. Firstly, phytoplankton may respond by immediate uptake of the nutrients which are then stored for later use. The phytoplankton, with their stored nutrients, are advected away from the fish farm site through water movement and may bloom away from the farm if environmental conditions (such as water temperature, climate and water movement) are optimal for growth (Smayda 2006). This would be a useful strategy if phytoplankton were nutrient limited. Phytoplankton in oligotrophic waters, such as the open ocean, are known to up-take and store nutrients as they move into a nutrient hot-spot (McCarthy 1977; McCarthy and Goldman 1979). Droop (1983) has shown that luxury consumption of nutrients other than phosphorus is common in phytoplankton. Thus, phytoplankton can up-take nutrients and store them for later use. Indeed, Eppley and Strickland (1968) concluded that growth rate is more closely related to the cellular content than to the external concentration and that variation in the cellular content of nitrogen and phosphorus observed in phytoplankton, represents the concentrations necessary to maintain given rates of growth imposed by other factors.

A study was conducted on nutrient status in phytoplankton in St Margaret's Bay, Novia Scotia (Guildford 1993). Measurements of N, Si and P, in this study, were made near aquaculture sites and at offshore sites, along a 2 km transect over a 4 day period. It was found that

phytoplankton at the aquaculture sites were less N deficient (percentages not given) than the offshore site and Si was taken up more rapidly. Guildford (1993) concluded that point sources of nutrients (mussel string and salmon cages) resulted in altered phytoplankton nutrient status; this would support the hypothesis that there is immediate uptake of nutrients of phytoplankton in the vicinity of a fish farm. This hypothesis may explain toxic nuisance blooms occurring at some distance from aquaculture sites in general. For example, there has been an alarming rise in the incidence of algal blooms and scallop poisoning events where scallop farming occurs in the neighborhood of fish farms in Scotland (Staniford 2002).

Phytoplankton may also respond through immediate uptake of the nutrients but in this case, if the environmental conditions are optimal for growth, then the phytoplankton will bloom locally. Most studies around fish farms have been concentrated on finding a localised link between fish farm waste and phytoplankton dynamics.

High concentrations of phytoplankton biomass (chlorophyll *a*) have regularly been recorded at fish farm sites (Pease 1977; Weston 1986; Caine 1989; Rensel 1989, 1990) and may be due to locally increased nutrient levels (hyper-nutrification). One of the earliest studies to attempt to determine the effects on the marine environment from fish farm activities was conducted by Pease (1977) at a small mariculture research facility in Henderson Inlet in Puget Sound. Water quality measurements, which included chlorophyll *a* (but not species composition or abundance), were made monthly from March through to December of 1974. Elevated

chlorophyll *a* levels were found at the net-pen sites, compared to controls. However, over the whole of the sampling period, which included the different seasons, no differences were found. It was concluded that this was normal for the area as higher levels of chlorophyll *a* had been recorded in other parts of the Inlet. Therefore, it was during the most productive season for phytoplankton, in this case spring, when there were fish in the pens, that significant differences were found around the net pens. This is important in itself as it demonstrates that, when there are fish in the pens, there are localised increases in chlorophyll *a.* Therefore, there is an effect, even though it disappears, which means that the effects begin locally and any nutrients which are not immediately taken up or diffused out of the water column are moved to some distant site.

Other studies have also found similar effects of chlorophyll *a* around fish pens. For example, the effects of a salmon-net pen farm (Rensel 1989) on dissolved nutrient concentration, phytoplankton density and growth rate were investigated in a shallow passage of southern Puget Sound. The data for this report was taken on one sampling day in May and one sampling day in June 1988 and elevated levels of chlorophyll *a* were reported at the net-pen sites prior to fish release during low tide. Concentration of ammonia was higher within the net-pens and total ammonia increased as a percentage of upstream ambient levels. The effect disappeared after the salmon were taken out of the pens and it was concluded that the input from the fish farms had no effect on the phytoplankton population. Taking two measurements is not statistically powerful so conclusions are difficult. However, from their results it appears

that there was a localised 'effect' on ammonia and chlorophyll *a.* Whilst the fish were in the pens, ammonia was high as was chlorophyll *a*, but the effect disappeared when the fish were removed.

Wildish *et al.* (1993) found no evidence that microalgal blooms were enhanced near salmonid farm sites in the Bay of Fundy. These sites are characterised by 6-m tides and rapid flushing >4 cm/s. In this instance no effect was seen, as the rapid flushing either removed the excess ammonia or associated algal production. Therefore, a great deal is dependent on the type of water body the fish farm is located in. These studies show that there are localised effects on chlorophyll *a* when there are fish in pens because when the fish are removed the effect measured disappears. The effect is not evident when chlorophyll *a* measurements are averaged out over the whole year, as the seasonally high measurements are balanced by the low measurements at other times of the year.

A third way that phytoplankton may respond to nutrient inputs is that the nutrients are moved away from the fish farm area by water movement. The phytoplankton uptake the nutrients at a distance from the farm and if conditions are optimal for growth there will be a bloom away from the farm. In this instance nutrients are moved away from the fish farm and up-take by phytoplankton occurs away from the farm. For example, in a study of water quality in Florida Bay, Brand (2002) has shown that nutrient rich water from Florida Bay is transported into the Florida Keys coral reef tract, where water quality has declined and algal blooms are now common. This has only recently been recognised as possible in large fish farm sites.

Finally, any changes in N and P ratios in the water column will increase phytoplankton blooms and/or alter species composition or diversity. It has been shown that a relationship does exist between blooms of phytoplankton and the N and P loads of coastal waters (Hodgkiss and Ho 1997), particularly in coastal waters where phytoplankton are nutrient limited. N and P loads in coastal waters are mostly derived from sewage discharge and stormwater run-off. Alterations, both long and short term, in the N:P ratio are accompanied by increased blooms of non siliceous phytoplankton groups (Hodgkiss and Ho 1997). Fish farm waste is similar to sewage discharge, in that it does not contain silicate, which is essential for diatom frustule formation. Diatoms are potentially unable to compete with other non siliceous types of phytoplankton which often include toxic species such as *Karenia brevis* or *Karenia mikimotoi*. Smayda (1990) has stated that there is increasing evidence worldwide, that the dissolved form and ratios of nitrogen and phosphorous released from fish farms can stimulate and promote phytoplankton growth with the potential for toxic species out-competing benign types.

Evidence for this has come mainly from laboratory experiments and freshwater systems. Nishimura (1982) showed the stimulating effect on *Gymnodinium aureolum* growth of yellowtail food and faeces extracts and seawater from fish farms. Arzul *et al.* (1996) extended the comparison of these effects to *Chaetoceros gracile*, *G.* cf. *nagasakiense* and *Alexandrium minutum*, and some effects were also investigated according to particular chemicals provided by aquaculture: nutrients, urea and organics. The study showed a complex relationship between the additives

and phytoplankton growth rates. Cell division took place in all trials except for some carried out on *A. minutum*. *C. gracile* was more often inhibited than stimulated. *G.* cf. *nagasakiense* was stimulated, at the same rate, by different concentrations. *A. minutum* was strongly inhibited by the products of fish culture. It was concluded that as well as an enrichment of phytoplankton growth, fish farming is likely to perturb both growth rates and hence species composition in phytoplankton. Drift towards fewer, or only single species (low species diversity), is likely to be one of the consequences, which would also favour the likelihood of bloom formation (Takahashi and Fukazawa 1982).

Low species diversity is thought to threaten ecosystem functioning and sustainability and this is exemplified in early studies on terrestrial ecosystems (e.g. Tilman *et al.* 1996, 1997). Studies demonstrated that as the number of species in communities increases, there is a complementary shift or increase in certain ecosystem processes, such as productivity, whereas decomposition may remain unaffected. Studies in marine coastal systems have revealed similar effects for diversity. For example, Emmerson *et al.* (2001) investigated diversity-function relations in mesocosms containing a gradient of species richness, using intertidal invertebrates. The function measured was the flux of nutrients to the overlying water column. Species pools were sampled from Scotland, Sweden and Boston Bay, South Australia. Emmerson *et al.* (2001) concluded that no global effects were evident due to site differences but their data showed that there is reduced variability (increased stability) in ecosystem function as diversity increases in a marine benthic ecosystem.

This is a strong indication that a drift toward fewer or less species may have, as yet undetermined and complex adverse effects on ecosystems whether terrestrial or marine

Changes to phytoplankton community composition have also been investigated. Studies performed on lagoon systems in the Po River Delta associated with intensive aquaculture demonstrated that waste effluent from intensive lagoon aquaculture had a profound effect on the lagoon ecosystem (Sorokin *et al.* 1999). This effect was demonstrated in the transformation of the phytoplankton community in two of the lagoons, which had experienced medium to high fertilization from fish farm effluent, by favoring domination of mixotrophic and/or toxic dinoflagellates, in this case *Alexandrium tamarense*. Although the Po River Delta is a shallow lagoon system and difficult to extrapolate to a deeper coastal marine environment, there remains the suggestion that changes to phytoplankton community composition occurs when nutrient ratios are altered through anthropogenic inputs into the environment.

However, in reality there is a complex of the all of the above scenarios operating at the any one time. Determining the transformation of phytoplankton community structure and biomass (chlorophyll *a*) is only possible through long term monitoring of phytoplankton distribution and abundance at ecologically relevant temporal and spatial scales. Regular monitoring of phytoplankton around fish farms is also necessary to alert authorities to the possibility of a harmful bloom occurring. This is because

toxicity from some phytoplankton can occur at very low numbers (sub-lethal toxicity).

Broad Aims

The objectives of this study were to determine the distribution and abundance of phytoplankton in Boston Bay and Spencer Gulf, particularly targeting potentially harmful algae; and to determine if there is a close association between tuna fish farms in Boston Bay and phytoplankton. The major hypotheses tested during this study were:

1. Phytoplankton abundance and biomass will be larger around tuna fish farms in Boston Bay and surrounds compared to control sites.
2. Potentially harmful algal blooms develop in Boston Bay rather than being brought in with currents from Spencer Gulf.
3. Temporal and spatial analysis of directional transects of in *vivo* fluorescence around tuna farms will reveal higher fluorescence signals around the fish farms than at control sites.

Chapter 2

Phytoplankton biomass, species composition, distribution and abundance around tuna fish cages in Boston Bay, Port Lincoln, and near- shore Spencer Gulf, South Australia

Introduction

In the past few decades there has been increasing concern with the eutrophication of coastal areas as a major factor causing ecosystem change (Environment Protection Authority 2003). Aquaculture, among other sources of anthropogenic waste, is described as a potential source of eutrophication in the coastal area (Pitta *et al.* 1999). Many studies have established that soluble nutrients, continually released into the water column from marine fish farm activity, are now adding to coastal hypernutrification (Pridmore and Rutherford 1992; Handy and Poxton 1993; Gowen 1994; Folke *et al.* 1997; Chen *et al.* 1999; ICES 1999; Edwards 2001; Navarro 2000; Arzul *et al.* 1996, 2002; Dosdat 2002; Tett and Edwards 2002; Environment Protection Authority 2003; Smayda 2006). Cloern (2001) also notes that intensive aquaculture in coastal waters disturbs sediments through unnaturally high concentrations and deposition of organic matter that alter sedimentary processes and oxygen concentrations.

Several studies have addressed the impact of phytoplankton on aquaculture, such as mortality to animals due to harmful algal blooms (Rensel 1989; Ono *et al.* 1996) and fish deaths due to decomposition of mass blooms of non-toxic phytoplankton (Shirota 1989). Prior studies around fish farms have looked for localised eutrophication effects from

nutrients, and have concentrated on measuring biomass in the form of chlorophyll *a* and counting phytoplankton numbers (Braaten *et al.* 1983; Gowen and Bradbury 1987; Rensel 1989, 1990; Foy and Rosell 1991; Beveridge 1996; Clarke 1996; Cartwright 1998; Clarke *et al.* 2000). Research has shifted to using groups of phytoplankton species and communities such as diatoms, dinoflagellates, flagellates and ciliates (Pitta *et al.* 1999) and nanoflagellates, ciliates and bacteria (Arulampalam *et al.* 1998; Navarro 2000) at fish farm sites as measures of eutrophication.

However, in Australia published information on the effects of fish farming on marine phytoplankton populations is limited. Tuna fish farming in Boston Bay began in the early 1990s after the collapse of the southern bluefin tuna (*Thunnus maccoyii*) wild fishery (Bureau of Rural Sciences 1993). Farmed tuna production has since increased from 3,362 t in 1995–96 to 5,080 t 2003–04 (EconSearch 2006). Currently in Boston Bay and Spencer Gulf there are approximately 60 active fish farms (PIRSA-Aquaculture Group 2006).

Information relating to phytoplankton in Boston Bay, comes only from industry consultancies and government reports (Deland and Jones 1973; Engineering and Water Supply Department 1976; Shepherd 1975; Department of Agriculture and Fisheries 1975; Caldwell Connell Engineers 1976; Harbison and Wiltshire 1993) or as a result of water quality monitoring programs (Clarke 1996; Cartwright 1998; Madigan *et al.* 2003). Current discussion centers on the idea that anthropogenic input of nutrients, including fish farm waste, will cause a change in the ratio of

nitrogen to phosphorous or silica in the aquatic environment and this will favour particular types of non siliceous phytoplankton and dinoflagellates (Smayda 1990; Hodgkiss and Ho 1997; Graneli *et al.* 1990) and lower phytoplankton diversity (Lehtinen *et al.* 1998; Leppakowski *et al.* 1999). Laboratory and fields studies have demonstrated that alterations in the community structure and diversity of phytoplankton in response to nutrient inputs are possible (Nishimura 1982; Arzul *et al.* 1996; Hickel 1998; Sorokin *et al.* 1999). Phytoplankton respond complexly to a multitude of factors in the marine environment, and they have single and overlapping habitat niches much in common with terrestrial vegetation communities. A recent model on the functional classification of species and habits may be useful in giving an ecological interpretation of species found in and around fish farm sites.

Functional classification of species and habitat

The functional classification model is based on terrestrial groupings of organisms in which each is a functional unit. Each unit is named after one or two species that are represented in a particular community-type (Reynolds *et al.* 2002). The term functional group distinguishes among phytoplankton on the basis of specialist adaptations and requirements (such as having a high affinity for ammonia or phosphorous at low external concentrations or of requiring skeletal silicon, or have good light attenuation capabilities) (Reynolds *et al.* 2002). Therefore, this type of grouping can offer a way of understanding and predicting the distributions and dynamics of natural populations of phytoplankton (Reynolds *et al.* 2002).

Smayda and Reynolds (2001) have developed a system of classifying phytoplankton into functional groups for the marine environment. Dinoflagellates involved in harmful algal blooms are used in their model. Smayda and Reynolds (2001) group significant organisms together as indicators of specific habitat conditions. In characterising communities and dynamics of phytoplankton, it was determined that this should rest on two basic selection features (Smayda and Reynolds 2001) – 'life-form and species-specific selection', taking into consideration that bloom species are usually selected as a result of being in the right place at the right time at suitable inoculum levels (Smayda and Reynolds 2001). These groupings are useful in determining characteristics of particular habitats under investigation. Using Smayda and Reynold's (2001) functional grouping model for dinoflagellates in this study may give some initial insight into phytoplankton 'habitat condition' in the Boston Bay, Spencer Gulf area.

Aims

The aim of the present study was to determine the distribution and abundance of phytoplankton species and biomass around tuna farms in Boston Bay and Spencer Gulf and to define the habitat requirements of the dominant bloom organisms.

Methods

In order to measure long term spatial and temporal trends in water quality near and far from tuna farms, water samples were taken every 6 to 8 weeks from 26 sites, including 10 tuna cages (TC), in Boston Bay and

Spencer Gulf, South Australia (Figure 1) over a period of 24 months from September 1997 to March 1999. The sampling region was also divided into four areas to give a regional perspective to the data as displayed in Figure 1 (area 1=west of Boston Island; area 2=Proper Bay, Spalding Cove including Bickers Island; area 3=east of Boston Island; area 4=upper Spencer Gulf; area 5=lower Spencer Gulf).

Areas considered most unaffected by tuna farming and anthropogenic influences were; the northern most part of the bay, Sites 10 and 10a; the outer eastern side of Boston Island, site 6; east and west of Rabbit Island, site 2 and 2a respectively; and Taylors Island, sites 3, 4 and 5. Site 1 was a tuna farm site positioned north of Rabbit Island in Spencer Gulf (SG) to show primarily the effect of a tuna farm in the Gulf area. Tuna farms were selected from the eastern and western side of Boston Island to show the effect of location as currents are much slower inside the island than on the outside. Sites in Proper Bay, where much of the anthropogenic inputs occur, were also sampled primarily to compare differences in biomass, hypothesising that there may be a larger effect in this area. Sampling of some sites on the eastern side of Boston Island was hindered because of weather constraints; therefore, not all sites were sampled on each field trip. All sites, including the Spencer Gulf sites are classified as near-shore environments as samples were taken within 500 m of the shoreline.

Figure 1: Map of Boston Bay (BB) and Spencer Gulf (SG), South Australia, showing, all sampling sites including tuna cages (TC) and controls (C) and areas used in the statistical analysis. Area 1=west Boston Island, area 2=Proper Bay, area 3=east Boston Island, area 4=upper Spencer Gulf and area 5=lower Spencer Gulf.

Water samples were collected with a Niskin Bottle from a depth of 1 m, 7 to 9 m and 1 m above the bottom of each site. Due to the homogenous nature of the bay (Paxinos *et al.* 1996) samples were pooled and sub-samples were then poured into one-litre bottles, half of which had been earlier prepared with 5 mL of acidified Lugol's iodine solution. One litre samples were filtered onto Millipore GS filters. Filters were immediately frozen and stored in darkness at -20°C for subsequent acetone extraction of chlorophyll *a* as outlined by Strickland and Parsons (1972). Data on nutrient concentrations came from samples taken at the same time by SA Water (SA Water unpublished data) and in unpublished reports (Clarke 1996; Cartwright 1998). Ammonia data was selected for use, as the main soluble waste product from fish farm activity is ammonia as nitrogen (N), (Gowen and Bradbury 1987; Rensel 1989; Rensel 1990) and phytoplankton are known to preferentially uptake ammonia (Fogg 1991).

Replicate phytoplankton counts were enumerated using the Utermöhl method (Lund *et al.* 1958). Particular live organisms and micrographs were used for identification and confirmed by Professor Gustaaf Hallegraaf, University of Tasmania. On one occasion cells were concentrated and sent to New Zealand to determine brevetoxin content.

Physico-chemical parameters of temperature, salinity, dissolved oxygen and pH were recorded at 3 depths (1 m, 7–9 m and 1 m above the sea-bed) simultaneously using a Hydrolab, DataSonde® 3 Multiprobe Logger. Visibility was measured using a Secchi disk.

Statistical analysis

A total of 131 taxa were sampled during this project, with many taxa occurring infrequently in the samples taken. This makes it difficult to look for treatment effects. The sheer number of potential comparisons then becomes enormous, creating severe difficulties for both Type I and Type II errors. In this study, a combination of Principal Components Analysis, MANOVA, non-parametric tests and t-tests were used to examine the species distribution and abundance of phytoplankton around tuna cages and controls. The study site was divided into 5 areas (Figure 1), area 1=west Boston Island, area 2=Proper Bay, area 3=east Boston Island, area 4=upper Spencer Gulf and area 5=lower Spencer Gulf, for the purpose of testing for regional effects.

Results

Water quality

One of the objectives of this study was to analyse trends in temporal and spatial variation in water quality associated with the tuna farms and areas where there were no tuna farms (controls). Since the spatial variation in temperature and salinity was not significant, only the mean values for all the tuna cages and controls were plotted in Figure 2a. Temperature followed the same seasonal pattern at tuna cages and controls, and there was little variation between surface and bottom temperatures. The mean average temperature was at a maximum in March (21.7^{o}C) and minimum in September (13.5^{o}C). Salinity followed the same seasonal pattern at tuna cages and controls, (Figure 2b) and there was little variation between surface and bottom salinities. Salinity ranged between 36.2 and 37.7. Recorded pH values (Figure 2c) ranged between 7.93 and 9.28 pH units and were the same at tuna cages and controls with little variation between bottom and surface values.

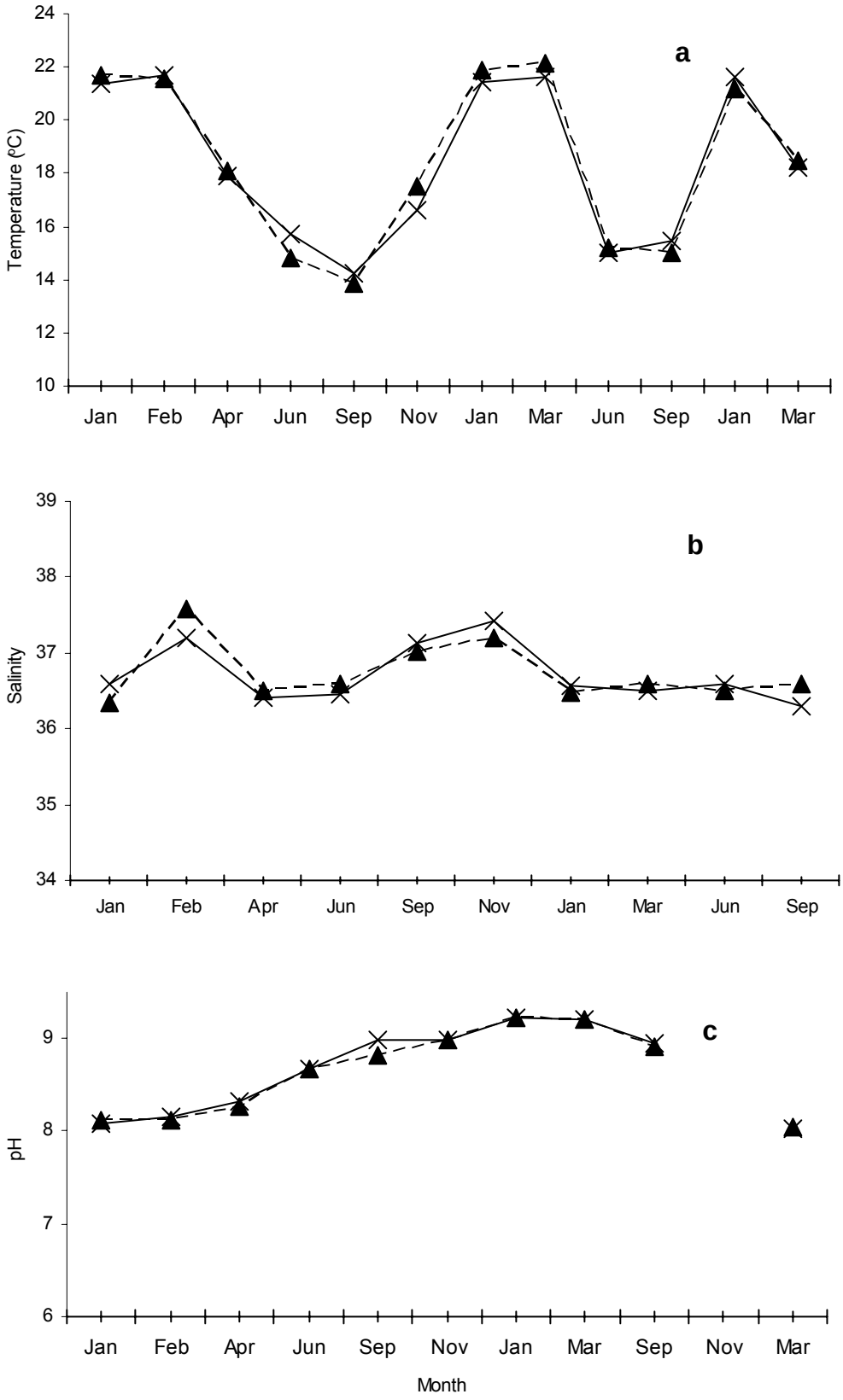


Figure 2: Mean (± 1SD) (a) temperature, (b) salinity, and (c) pH at tuna cages and controls, 1997–99.

Dissolved Oxygen

Dissolved oxygen (DO) (Figure 2d) ranged between 6.4 and 10.2 mg/L with average levels at tuna cages (TC) and controls (C) of 7.3 and 7.6 mg/L respectively. There was no significant difference between DO levels at the tuna cages and controls (p<0.01, n=46TC, n=43C). DO levels decreased generally at all sites in the summer months and increased from June to November. There was very little difference between the surface and bottom DO throughout the year.

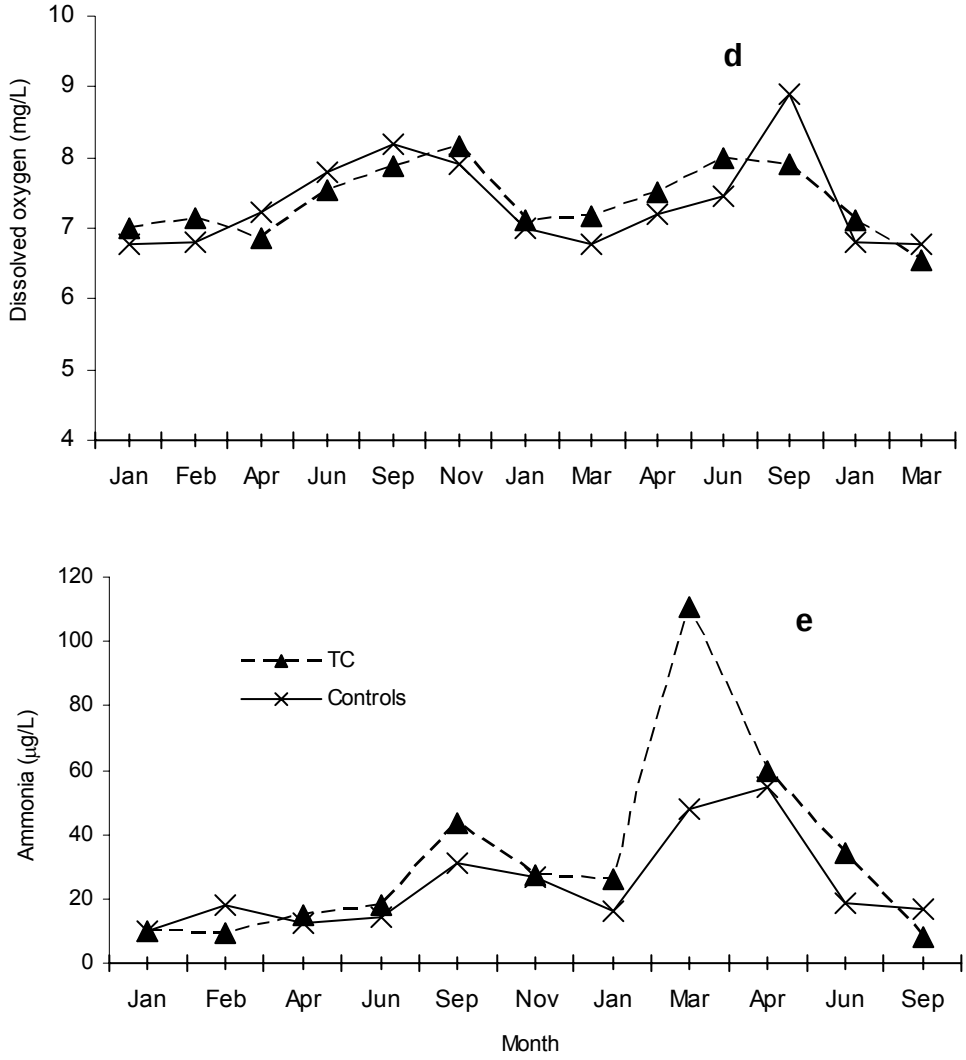


Figure 2: Mean (± 1SD) (d) DO, and (e) ammonia at tuna cages and controls, 1997-99.

Ammonia

Ammonia concentrations ranged between 5.0 and 245 μg/L at tuna cages (TC) and 5.0 and 112.0 μg/L at controls (C) however these were not statistically significant (p<0.05). Ammonia at all stations followed a seasonal pattern that was opposite that of DO (Figure 2e). There was a general increase in ammonia overall during the months of autumn in 1997/98.

Chlorophyll *a*

Chlorophyll *a* (Chl *a*) levels generally peaked from March to June. Chl *a* levels for all Spencer Gulf sites were low compared to Boston Bay sites for most of the year. Chl *a* at the Spencer Gulf sites ranged from 0.12 to 1.10 µg/L. For Spencer Gulf sites over all average levels for the year were consistently highest at SG1/TC, 0.69 µg/L and lowest at SG3 and SG4, 0.54 µg/L. Chl *a* levels for the eastern side of Boston Island ranged from 0.15 to 2.35 µg/L. Generally, levels were higher at BB8/TC, BB8a and BB9/TC east of Boston Island In 1999 figures were recorded at >5.0 µg/L. Chl *a* levels for the western side of Boston Island and the Northern Channel ranged from 0.19 to 1.45 µg/L. Only one cage had fish in it all year and this was BB12/TC (Research and Development site for SARDI-Aquatic Sciences). The cages at this site are smaller than normal cages and held smaller amounts of fish. Chl *a* levels for Proper Bay, Spalding Cove and the southern channel ranged from 0.20 to 1.86 µg/L.

To check this further, the mean of the chlorophyll data was calculated and plotted against sample dates and treatment and controls. The

resulting plots show a large deviation from the mean chlorophyll (± 1SE) calculated across all sites and for each sampling date, for treatment and controls, which were due to a few outliers. The data was therefore transformed using LOG10, Ln and a square root transformation. LOG10 transformation appeared to improve the spread of the data; however, the large deviation from the mean is still evident in some samples. Figure 3 shows mean chlorophyll (LOG10, ± 1SE) at all sites at tuna cages and controls; there appears to be some difference between tuna cages and controls.

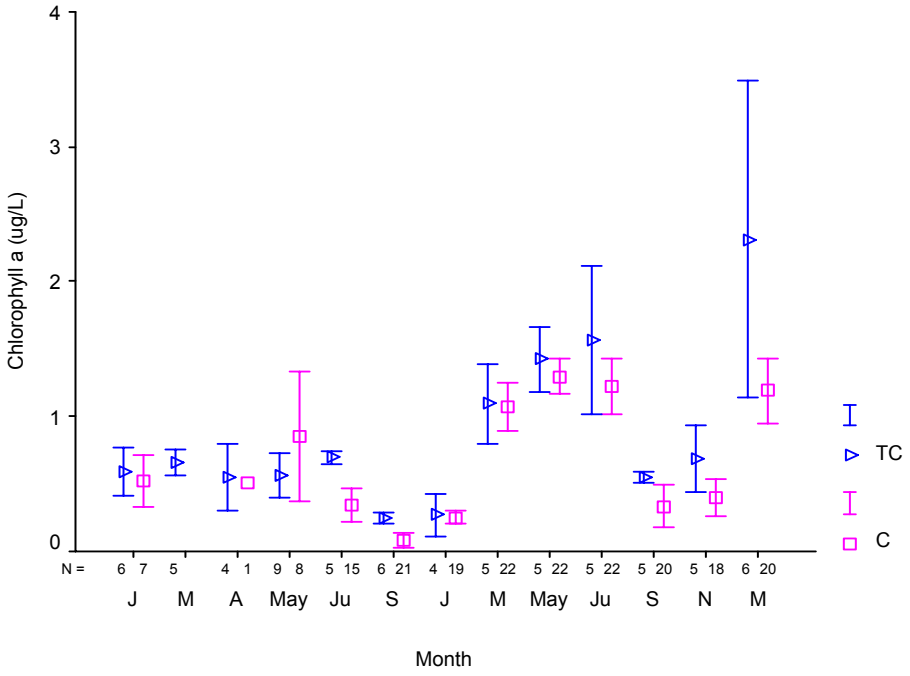


Figure 3: Mean chlorophyll *a* LOG10 (± 1SE) calculated across all sites and each sampling time, for treatments (TC) and controls (C).

Statistical analysis

Univariate analysis was used to investigate the effects of chlorophyll between area, years, sampling date, treatment and controls. However,

Levene's test was significant (p<0.001), indicating that the assumption of equal variances is not met and a non-parametric approach was necessary. The data were grouped by sampling date, for treatment and controls. The existence of chlorophyll effects was assessed using a Kruskal-Wallis non-parametric ANOVA. This involved a total of 13 comparisons for each date. Because a large number of unplanned tests were performed, a sequential Bonferroni adjustment (Rice 1989) was applied to account for any Type I or II error that may have occurred. In this case with 13 comparisons, α=0.05/13 therefore p<0.003. Statistical significance ranged from p<0.006 to p<0.827. This analysis showed that there was no significant effect from treatment and controls within each sampling date.

To test for within year effects of chlorophyll for treatments and controls, the mean (LOG10) chlorophyll levels were grouped and a Kruskal-Wallis non-parametric ANOVA performed. No significant effect was found within years from 1997 to 1999 (p=1.000) for treatments and controls.

To test for between year effects, the mean (LOG10) chlorophyll levels were grouped for each year and the existence of chlorophyll effects was assessed using a Kruskal-Wallis non-parametric ANOVA. The results showed a significant effect of chlorophyll between the years 1997 to 1999 (p<0.002). To test between means of each year Independent T-tests were performed and a significant effect was found between the years 1997 and 1998 (p<0.001).

Area effects between treatments and controls

To test for between area effects, the mean (LOG10) chlorophyll levels were grouped for treatments and controls for each area and the existence of chlorophyll effects was assessed using a Kruskal-Wallis non-parametric ANOVA. The results showed a significant effect of chlorophyll for area (p=0.012). To test between means of each area, Independent T-tests were performed and a significant effect was found between the lower Spencer Gulf sites and all other sites (p<0.004).

Phytoplankton abundance and distribution

Principal components analysis

A total of 131 taxa of phytoplankton were identified (Appendix 1): only the 14 dominant taxa were used (Table 1) as they accounted for approximately 60% of the variation in this analysis. Taxa that had rare or sporadic representation were also examined, but on a case-by-case basis, to see if their occurrence may have been influenced by treatment.

Table 1: Taxa of the 14 dominant phytoplankton used in the principal components analysis, with abbreviation and the principal component number.

Component number	Taxa (abbreviation)	Taxa
1	Cc	*Cylindrotheca closterium*
2	Ch	*Chaetoceros* spp.
3	Dmed	*Dactyliosolen mediterraneus*
4	Gb (Kb)	*Gymnodinium breve* (now *Karenia brevis*)
5	Gm (Km)	*Gymnodinium mikimotoi* (now *K. mikimotoi*)
6	Gs	*Guinardia striatum*
7	Gym	*Gymnodinium* spp.
8	Gyro	*Gyrodinium* spp.
9	Ld	*Leptocylindricus danicus*
10	Lmin	*Leptocylindricus minimus*
11	Nav	*Navicula* spp.
12	Nl	*Nitzschia longissima*
13	Ps	*Pseudonitzschia* spp.
14	Tery	*Trichodesmiun erythaeum*

Interpreting the factors

Table 2 shows that the cumulative percentage of variation explained by the four principal components extracted is 59.8%. The first PC explains 22.1%, PC2 explains 20.5%, PC3 explains 8.8% and PC4 explains 8.0%. In this analysis only 4 PCs were extracted.

Table 2: Loadings of each included taxon for the four principal components extracted and percent variation explained.

Taxa	**Principal Components**			
	1	2	3	4
Cc	-0.04139	0.89805	0.03091	-0.09181
Ch	-0.06804	0.71606	0.16170	0.08145
Dmed	-0.13518	-0.14964	0.58816	0.48162
Gb	0.84171	-0.16717	0.07323	-0.13309
Gm	-0.21085	0.15111	0.70126	-0.01898
Gs	-0.14550	0.01388	-0.45386	0.01617
GYym	0.84838	0.11847	0.12278	0.02109
Gyro	0.74414	-0.05607	-0.02514	-0.14987
Ld	0.02396	0.71928	0.05530	0.02119
Lmin	-0.00101	0.14320	0.23599	-0.08159
Nav	0.89058	-0.02388	-0.07076	0.14283
Nl	0.02528	0.06085	-0.11009	0.89348
Ps	-0.08451	0.95893	0.00336	-0.00775
Tery	0.46995	-0.05613	-0.27567	0.11939
% of Variance	22.104	20.533	8.884	8.060
% Cumulative Variance	22.104	42.637	51.521	59.581

The first 2-6 taxa with the highest positive/negative loadings for each of the four components are used for this interpretation. The principal components appear to contrast those taxa that bloom together because of specific habitat conditions. On inspection of factor loadings it appeared that the four principal components may have been related to taxa suites that varied in their time of blooming. To check this, factor scores were plotted against sample dates. The resulting plots are summarised as mean factor scores (± 1SE) calculated across all sites, for each sampling date, for each of the first three factors. Figure 4 shows that mean factor scores for the first PC relate to taxa that predominantly bloomed during March of 1998. There was no difference between treatment and controls in relation to taxa abundance; however, counts for controls show more variation as displayed by the error bars.

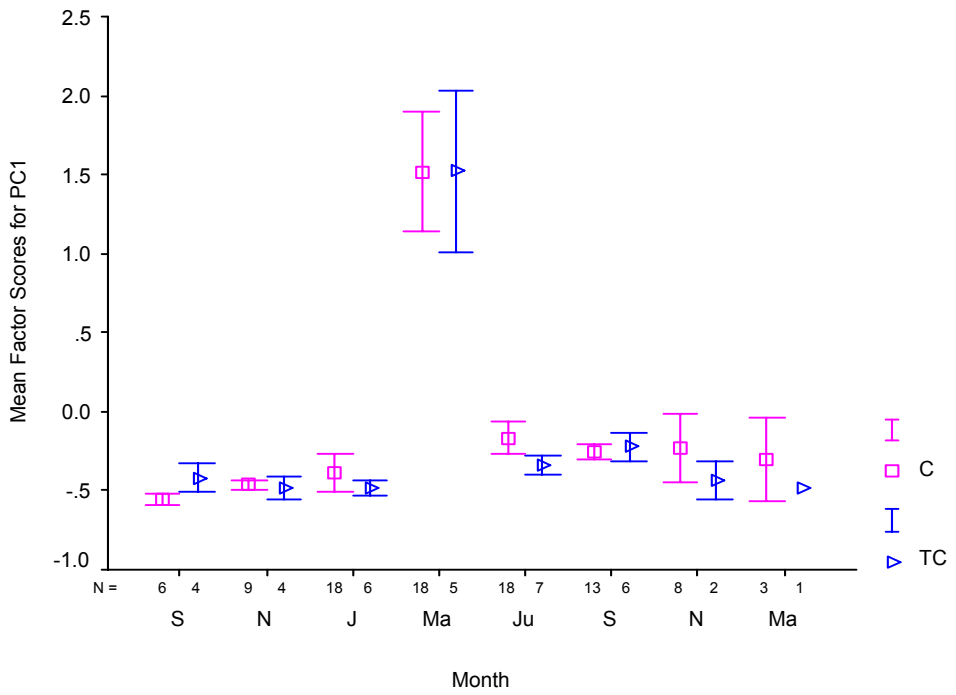


Figure 4: Mean (± 1SE) factor scores for PC1 and date for tuna cages (TC) and controls (C) in Boston Bay and Spencer Gulf.

Figure 5 shows that mean factor scores for the second PC relate to taxa that predominantly bloomed in June of 1998 with variation in counts being similar for both treatments and controls.

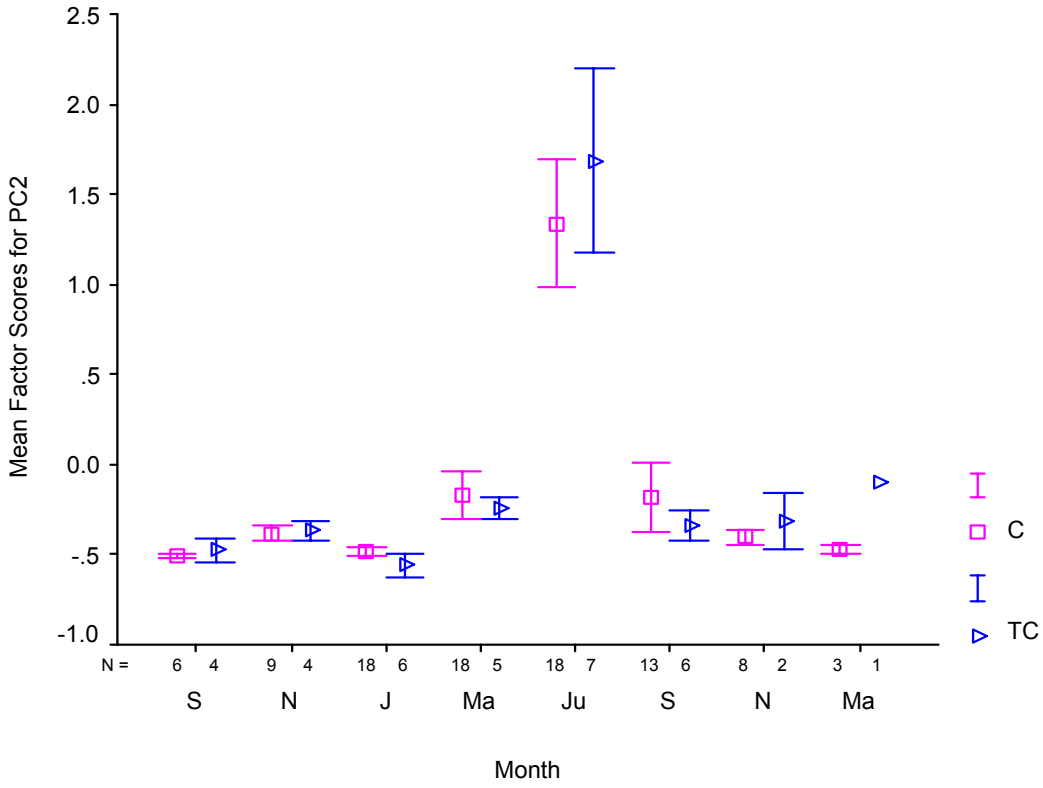


Figure 5: Mean (± 1SE) factor 2 and date, for tuna cages (TC) and controls (C) in Boston Bay and Spencer Gulf.

Figures 6 and 7 show the mean factor scores for PC3 and PC4 respectively. PC3 relates to taxa that predominantly bloomed in September, November, March and September again; some differences also appear between treatments and controls. PC4 results are somewhat variable but may relate to taxa that predominantly bloomed at controls in March and at treatments in September.

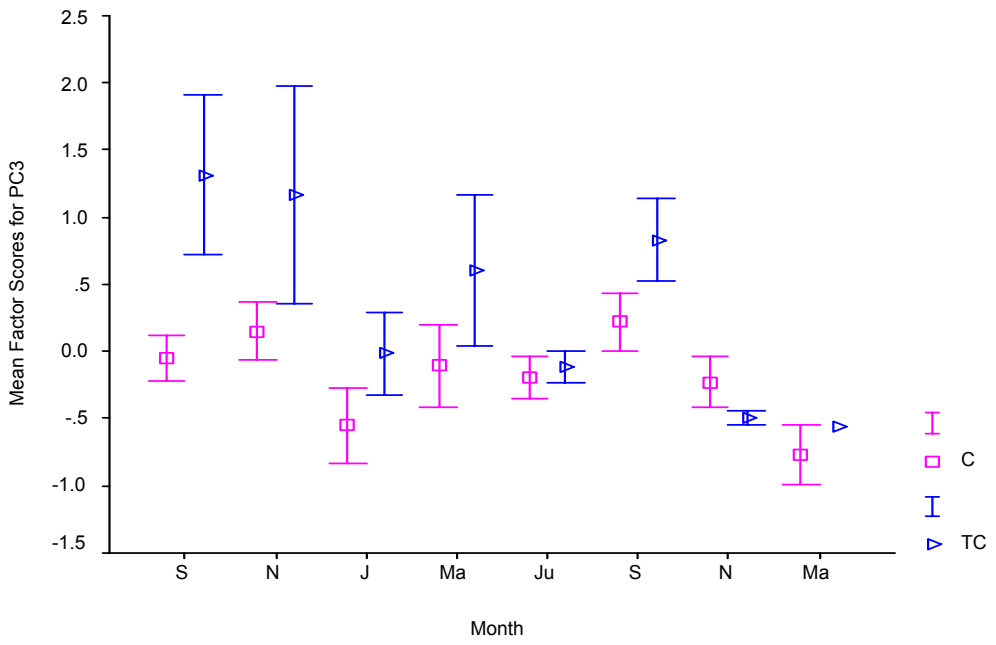


Figure 6: Mean (± 1SE) factor scores for PC3 and date, for tuna cages (TC) and controls (C) in Boston Bay and Spencer Gulf.

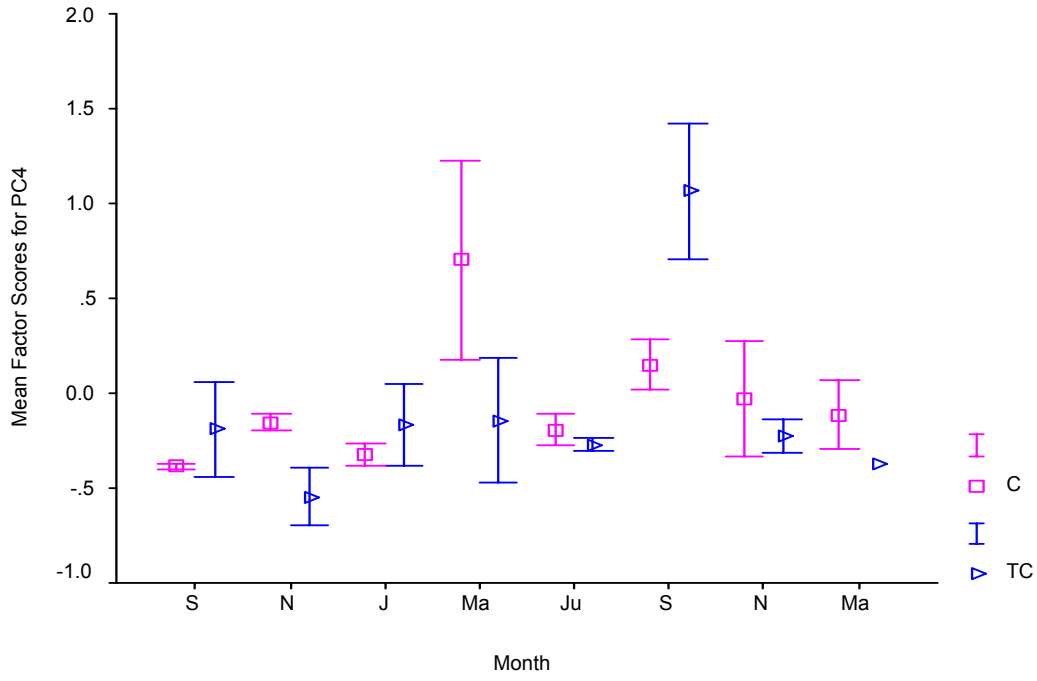


Figure 7: Mean (± 1SE) factor scores for PC4 and date, for tuna cages (TC) and controls (C) in Boston Bay and Spencer Gulf.

The first principal component (PC1) appears to have its major positive loadings from taxa that have gradually or abruptly increased in numbers from September 1997 and completed their bloom cycle sometime between March/June 1998 and negative loadings from those that correspondingly have increased in number until January and then September 1998.

The highest positive loadings for PC1 were 11, 7, 4, and 8 (Figure 8). With the exception of 11 a diatom, all of these taxa are small to intermediate sized dinoflagellates. Taxon 4 is an unarmoured dinoflagellate *Gymnodinium breve* (now *Karenia brevis*), taxon 7 and 8 were *Gymnodinium* spp., a large group of small, unidentified gymnoids and *Gyrodnium* spp. respectively.

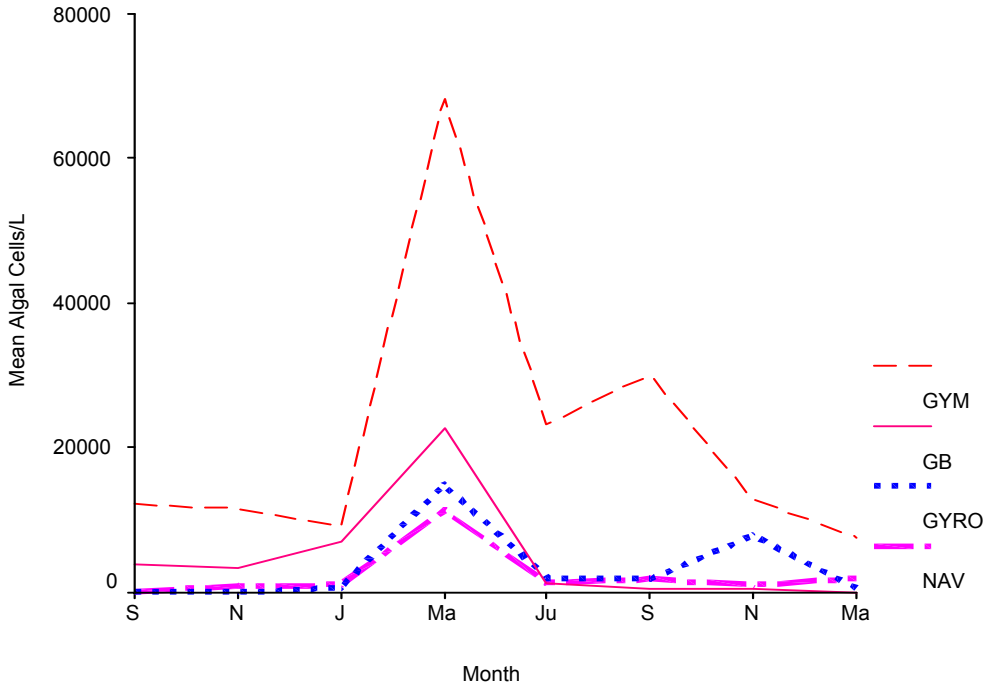


Figure 8: Mean algal cells/L of taxa positively associated with PC1. GYM=*Gymnodnium* spp.; GB=*Karenia brevis*; GYRO=*Gyrodinium* spp.; NAV=*Navicula* spp.

The highest negative loadings of taxa from PC1 were 5, 6, and 3. Taxon 5, *Gymnodinium* cf. *mikimotoi* (now *Karenia mikimotoi*) is an ichthyotoxic unarmoured dinoflagellate. Taxa 6 and 3, the neritic diatoms *Guinardia striata* and *Dacytiliosolen mediterraneus,* are also negatively associated with PC1 and bloomed at a similar time to *G. mikimotoi* (Figure 9).

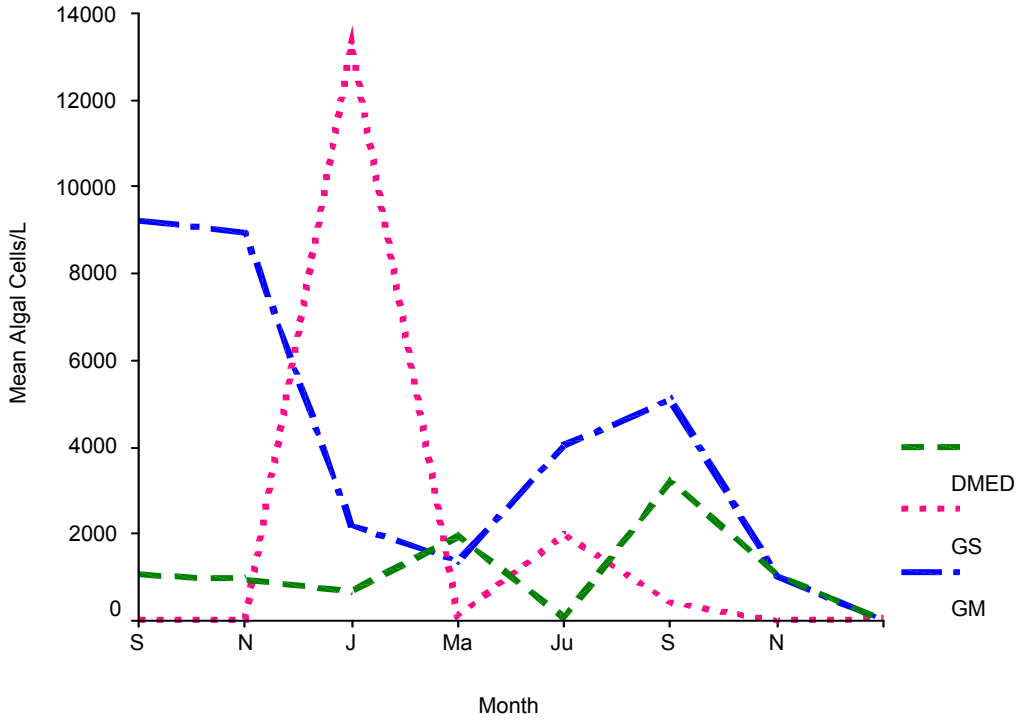


Figure 9: Mean cells/L of taxa negatively associated with PC1. DMED=*Dactyliosolen mediterraneus*; GS=*Guinardia striata*; GM=*Karenia mikimotoi.*

The positive loadings bloomed significantly (>4.0x10^4 cells/L) in March 1998 whereas the negative loadings for PC1 had lower cell counts (<2.0x10^4 cells/L) and bloomed significantly from November to January.

The highest positive loadings for PC2 were from taxa 13, 1, 9 and 2 (Figure 10). These taxa are the diatoms *Pseudonitzschia* spp,

Cylindrotheca closterium, Leptocylindricus danicus and *Chaetoceros* spp. respectively. The highest negative loadings for PC2 were from taxa 4 and 3, the unarmoured dinoflagellates, *K. brevis,* and the diatom *Dactyliosolen mediterraneus*. Taxa from positive loadings bloomed significantly (>2.0-5.0x10^4 cells/L) in June 1998 whereas taxa from negative loadings for PC2 had lower cell counts (<1.0x10^4 cells/L) and bloomed significantly in January.

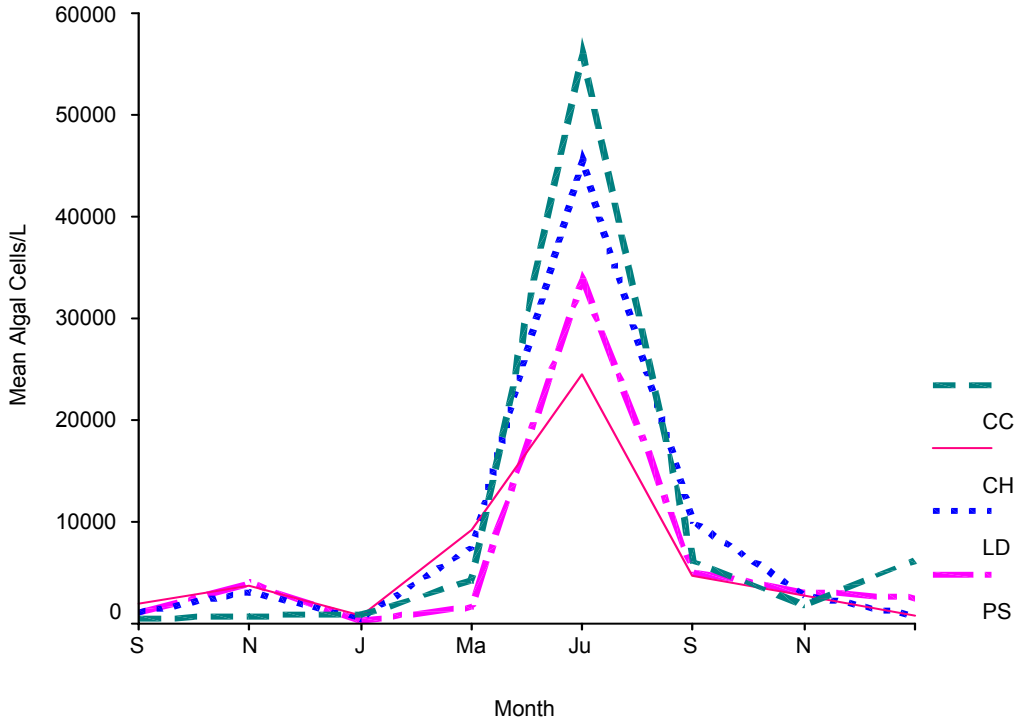


Figure 10: Mean algal cells/L of taxa positively associated with PC2. CC=*Cylindrotheca closterium,* CH=*Chaetoceros* spp.; LD=*Leptocylindricus danicus*; PS=*Pseudonitzschia* spp*,*

The highest positive loadings for PC3 (Figure 11) were from taxa 5 and 3, the unarmoured dinoglagellate, *K. mikimotoi* and the diatom *Dactyliosolen mediterraneus* respectively. The highest negative loadings

for PC3 (Figure 11) were from taxa 6 and 14, the diatom *Guinardia striata* and the cyanobacterium *Trichodesmium erythaeum*. The positive loadings appear to have bloomed significantly (<1.0x10^4 cells/L) from June to November whereas the negative loadings for PC3 (6.0x10^3-1.3x10^4 cells/L) bloomed significantly in January and March respectively.

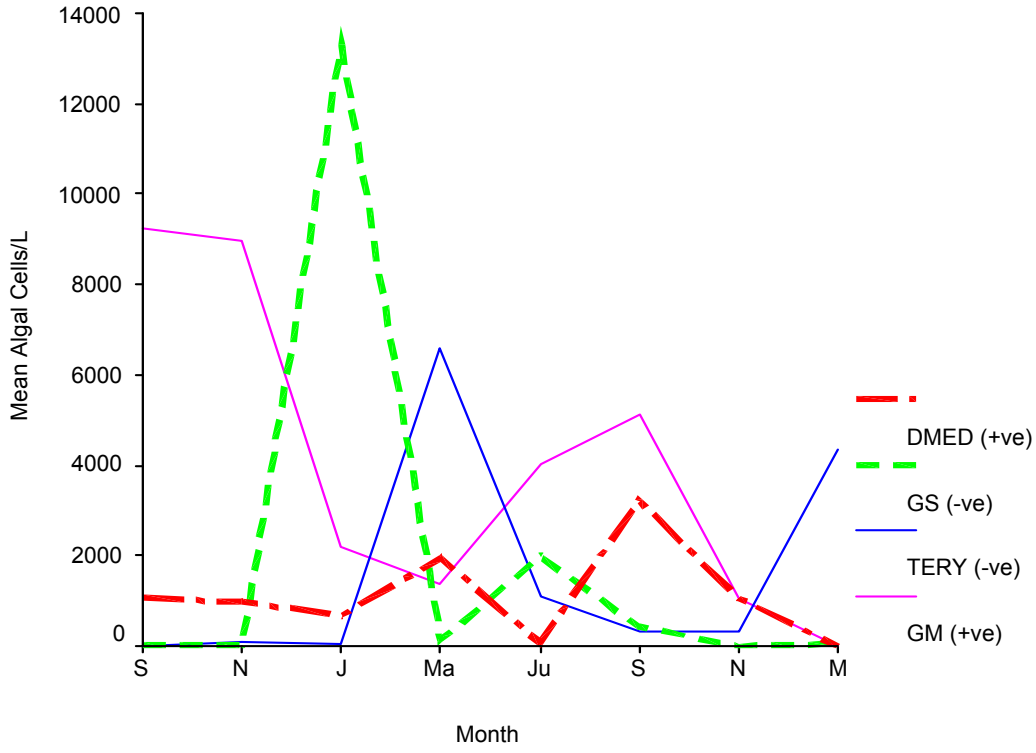


Figure 11: Mean algal cells/L of taxa positively (broken line) and negatively (unbroken line) associated with PC3. DMED= *Dactyliosolen mediterraneus*; GS=*Guinardia striata*; TERY=*Trichodesmium erythaeum*; GM=*Karenia mikimotoi.*

The fourth principal component (PC4) (Figure 12) appears to have its highest positive loadings for PC4, which were taxa 12 and 3, the diatoms *Nitzschia longissima* and *Dactyliosolen mediterraneus* respectively. The

highest negative loadings for PC4 (Figure 12) were from taxa 8 and 4, the dinoflagellates *Gyrodinium spp*. and *K. brevis,* which bloomed in March and November although it appears that *K. brevis was* disappearing from samples in November of 1998.

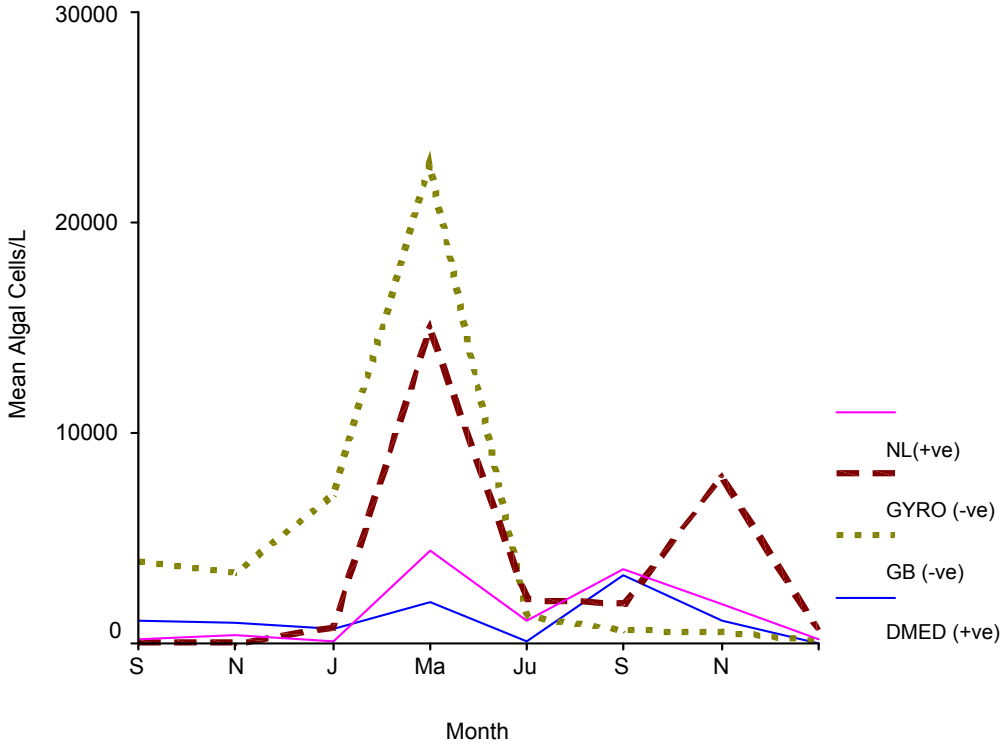


Figure 12: Mean algal cells/L of taxa positively (unbroken line) and negatively (broken line) associated with PC4. NL= *Nitzschia longissima* GYRO=*Gyrodinium* spp.; GB=*Karenia brevis*; DMED=*Dactyliosolen mediterraneus*.

Testing for area/treatment/seasonal differences

Because there were a large number of combinations of areas, dates and treatments, I started by investigating the existence of area effects (visual inspection of data strongly suggested seasonal effects). Data were grouped into treatment (tuna cages) and control samples for each

sampling date and the existence of area effects, using the extracted Principle Components within each of these combinations, was assessed using a Kruskal-Wallis non-parametric ANOVA. This involved a total of 15 comparisons for each principal component. Because of the large number of unplanned tests, a sequential Bonferroni adjustment (Rice 1989) was applied to account for any Type I or II error that may have occurred. In this case with 15 comparisons, $\alpha=0.05/15$ therefore $p<0.003$. Statistical significance ranged from $p<0.007$ to $p< 0.861$. This analysis showed that there was no significant effect from area and phytoplankton counts for each sampling period.

An Independent-Samples T-test using principal components 1-4 compared the means of phytoplankton counts to account for treatment effects. The data were grouped by date and then used 'numtreat (01)' as the grouping variable (0=tuna cage; 1=control). Statistical significance ranged from $p<0.021$ to $p<0.924$. T-tests showed no significant effect from treatment and controls and phytoplankton counts for each sampling period (date) ($p<0.003$).

Manova

Factor loadings were used in the MANOVA model in their original scale to test for area, treatment and date effects. The Box's M test was significant ($p<2.7\text{x}10^{-6}$) indicating that the assumption of equal covariance matrices is not met. If the sample sizes were similar then unequal variances would not have as large an effect on the MANOVA results; however, this data has sample sizes ranging from 1–18. The residual

plots, in particular the predicted values versus the residuals, indicate that there may be a positive relationship between means and variance for some of the dependent variables. This suggests that a variance stabilising transformation may improve the model fit. Taking the square root of the factor scores (plus a constant to give positive values) still gave a significant Box's M test ($p<4.3x10^{-4}$), even though the residuals seem to show an improvement in the variability for most of the dependents. The MANOVA shows a non-significant interaction between treatment and date, a non-significant treatment effect and a significant date effect. The log transform of the factors was also attempted but it did not improve the variability as much as the square root transformation did. Therefore the MANOVA showed that there was no effect between treatment and controls; however, there was a significant effect by season.

Abundance and distribution of phytoplankton

A total of 131 taxa were identified in this study (Appendix 1). A complete analysis of the community ecology of these organisms is not attempted here; rather a summary of the most dominant organisms and habitat preferences is offered. Picoplankton was often the most abundant organism found. Counts for picoplankton were generally several orders of magnitude higher ($>1x10^{-6}$ cells/L) than counts for other organisms.

Table 3 shows the mean counts (displayed as a percentage) by area, of phytoplankton groups, when picoplankton were excluded from the analysis. Lower Spencer Gulf was quite different from other areas as diatoms made up 74% of the phytoplankton community and dinoflagellates

21%, whereas in other areas dinoflagellates made up for >41% of the total. Dominant organisms for lower Spencer Gulf sites were *Guinardia striata*, *Chaetoceros* spp*. Nitzschia longissima, Pseudonitszchia* spp*., Gymnodinium* spp*., Navicula* spp*., Cylindrotheca closterium, Leptocylindricus* spp*.,* and *Licmorphia* spp*.*, compared to Boston Bay sites where *Gymnodinium* spp*., K. brevis, Cylindrotheca closterium, Chaetoceros* spp*, Leptocylindricus* spp*., Navicula* spp*., Gyrodinium* spp*.,* and *K. mikimotoi* were the dominant organisms*.* In general, for the group of *Gymnodinium* spp., the smaller dinoflagellates, such as *Heterocapsa* spp*.,* *Torodinium* spp., and *Katodinium* spp*.,* were most common. Potentially toxic species identified in this study included *Karenia brevis, K. mikimotoi, Dinophysis acuminata, D. acuta, Heterosigma* spp*, Ceratiurm fusus, Pseudonitzschia* spp., *Chattonella marina* and *Alexandrium minutum*.

Table 3: Mean cell counts (cells/L) for all phytoplankton groups (when picoplankton are excluded) displayed as a percentage by area.

Area	Dinoflagellates (%)	Diatom (%)	Other Flagellates (%)	Raphidophyte (%)	Blue green (%)	Total (%)
Proper Bay	44.93	50.92	2.0	0.33	1.83	100
Lower SG	20.69	74.71	2.3	-	2.3	100
Upper SG	41.3	57.14	1.56	-	-	100
East Boston Island	53.47	42.03	2.81	0.38	1.31	100
West Boston Island	42.4	52.69	2.46	0.77	1.69	100

Rare species with tropical to subtropical habitat preference identified in this study included *Podolampas palmipes, Climacosphenia* sp. *Stephanopyxis* sp., *Mastolgia* sp., and *Chaetoceros coarctatus*.

Discussion

Impact on water quality

The aim of this study was to determine if phytoplankton abundance and biomass was larger around tuna fish farms in Boston Bay and surrounds compared to control sites. The physical water quality parameters followed seasonal patterns and do not need to be discussed further. Average ammonia concentrations were generally higher in 1998 compared to average levels from previous reports in Boston Bay during 1996/97; <5.0–14.0 µg/L (Clarke 1996) and from 28.0–90.0 µg/L (Cartwright 1998) respectively. However, ammonia was not significantly different between tuna cages and controls due to uptake by phytoplankton and water movement dispersing the nutrient loading from the cages to surrounding waters. A study of fish farm sites (Pitta *et al.* 1999) in the Mediterranean has shown that ammonia concentrations at fish farm sites generally increased during feeding times and resulted from limited water circulation at the center of the farm where samples were taken. Current velocity may be reduced by 65% inside the cage (in Iwama 1991) due to the presence of one cage only. This reduction in water movement may explain generally higher ammonia levels detected on some occasions at the tuna cage sites.

DO concentrations were generally opposite that of ammonia during the autumn months. Phytoplankton blooms are most obvious in the autumn months in the Boston Bay area. It has been observed that oxygen depletion is mostly due to consumption in the water column through plankton community respiration and remineralisation (Chapelle *et al.* 1994). DO concentrations and chlorophyll *a* trends were similar (Figures

2d and 3), which indicates that oxygen concentration was being produced by photosynthesis (mostly phytoplankton) at a similar rate to that being consumed. Chlorophyll *a* levels were within the range for oligotrophic waters in a temperate environment only at the Spencer Gulf sites for most of the year (ANZECC 2000 guidelines for South Australia estimate an average of 1 μg/L).

Overall, there appeared no statistically significant effect of chlorophyll *a* between tuna cages and controls in Boston Bay or surrounds. However, there was a significant effect of chlorophyll *a* between the area of lower Spencer Gulf, where there were no tuna farms, and all other areas. There was also a significant effect of chlorophyll *a* between years and between seasons; this effect was observed most strongly on the eastern side of Boston Island. The observed increase in chlorophyll concentrations between years most likely represents a response to the progressive increase in ammonia concentrations. Karentz and Smayda (1984) observed that the initial response of an ecosystem to nutrient enrichment may be an increase in phytoplankton abundance; this may or may not be reflected in chlorophyll biomass depending on the amount of pigment present in the dominant organisms.

The apparent increase in chlorophyll *a* is surprising considering the eastern side of Boston Island is deeper than the western side and has strong currents and considering that chlorophyll *a* results for the eastern side of Boston Island have been consistently low up until 1998 (Caldwell

Connell Engineers 1976; Clarke 1996; Paxinos *et al.* 1996*;* Cartwright 1998).

In areas with a rapid flushing time, it has been suggested that there is limited uptake of fish farm wastes because phytoplankton are not present long enough to capitalise on the high production of nutrients (Gowen *et al.* 1992). Collos (1980) determined that if the diatom *Phaeodactylum tricornutum* is in a nitrogen limited environment for periods of between 1 and 5 days, the lag in nitrogen uptake mobilization increases from 30 to 60 minutes but for shorter periods of starvation (2–4hrs) there is no lag in mobilization of the uptake of nitrogen. Raimbault *et al.* (1990) found that *P. tricornutum* has an extremely high affinity for nitrate at very low concentrations (<1µM) and can rapidly uptake >50% of small nitrate supplies at an initial elevated level. Fogg (1991) reports that nutrient-depleted algal cells may take up ammonium ions extremely rapidly and experiments in culture show that appreciable amounts can be absorbed during brief exposures to high concentrations (in Fogg 1991). This is highly species dependent.

A fish farm cage in Boston Bay is 50 m in diameter. Average currents in Boston Bay and the Southern Channel range from <2.5–12.2 cm/sec (1.5–7.3 m/minute) respectively. Nitrogenous plumes have been reported at salmon fish farm cages, which are 15 m in diameter, of at least 30 m (Environmental Assessment Office 1996). Therefore, a nitrogenous plume at a tuna cage, which is 50 m in diameter, may conservatively extend for 100 m from the cage. In this case it would take a nonmotile diatom

anywhere from 100 minutes to 20 minutes to move through the cage/plume area and this may be sufficient time for uptake to occur. Furthermore, where there are over 60 fish farm sites, there will be considerably more than one nitrogenous plume for phytoplankton to move through.

Future examination of the nutritional status of phytoplankton would give a better indication of differences, if there are any, in uptake of nutrients by phytoplankton in and around fish farm sites and away from them. A study in Margaret Bay, Nova Scotia, of the nutritional status of phytoplankton in and around fish farm sites, found no spatial patterns in ambient concentrations of nutrients or chlorophyll (Guildford 1993). However, it was shown that point sources of nutrients, from mussel strings and salmon farms, resulted in a different phytoplankton nutritional status compared to communities away from the nutrient source (Guildford 1993).

Other physical forces combine to keep phytoplankton in the water column and to exploit particular niches. For example, for a nonmotile diatom such as *P. tricornutum* in the water column, these forces include vertical diffusion and the upwelling movement of water. Flagellates use other strategies: they are able to swim up and down the water column on a daily basis to take advantage of the nutrients near the seabed during the night and move back into the euphotic zone during the day to utilise the light for photosynthesis. For example, the dinoflagellate *Gymnodinium sanguinium* has a swimming speed as high as 26.4 m/day (Cullen and Horrigan 1981). If the swimming speed is greater than the speed of the

water movement then the organism is able to reproduce and bloom in that same place (Yamamoto and Okai 2000). Flagellates will bloom even in very turbulent conditions by using their swimming ability, whilst diatoms form blooms in the same place by their high growth rates with the aid of vertical diffusion and the upwelling movement of water (Yamamoto and Okai 2000). Therefore if fish farm plumes combine and the nitrogenous waste is not isolated at the fish farm cage then phytoplankton are moving through a hypernutrified area and will have sufficient time for uptake of nutrients and may subsequently bloom locally.

Another possible mechanism for increased chlorophyll *a* concentrations around fish farms may come from the biomass supported by tuna cage nets. Tuna cage nets support large fouling communities, comprised mainly of *Ulva*, which have been estimated for an entire tuna cage net to be approximately 6.5 tonnes (Cronin *et al.* 1999). Ten tuna cages therefore may hold approximately 65 tonnes of biomass at any one time. The biomass held by the tuna cage nets may regularly shed into the water column and become 'drift'. There is the possibility that when water samples are taken near the cages that some of this 'drift' is also taken into the water samples and cause fluctuations in the readings of chlorophyll *a*. However, drift cannot account for any temporal increases in chlorophyll *a*.

The difference in years for chlorophyll found in this study may also be described by natural variation of phytoplankton communities in the sea. Long-term studies of phytoplankton populations have revealed that abundance and composition may vary at the same place for short periods

of time (i.e. 5 years) (Karentz and Smayda 1984), which may or may not be reflected in phytoplankton biomass depending on the organisms present.

Principal components analysis

Principal component (PC) analysis extracted fourteen dominant phytoplankton species and groups found during the time of this study. Overall the PC analysis showed that there was no statistically significant effect from phytoplankton counts between fish farm sites and controls. The main effect found was from season and area.

The PC analysis was useful because it determined which organisms bloomed together, at which times of the year and at which sites. From these results a picture emerged of the dominant organisms found during this study, which made it simpler to apply the 'functional grouping' model for dinoflagellates to determine the habitat preference of these organisms. Of the taxa positively associated with PC1, taxa 4, *Karenia brevis* an ichthyotoxic dinoflagellate is common in the Gulf of Mexico and dispersed via the Gulf Stream (Geesy and Tester 1993). *K. brevis* is classified by Smayda and Reynolds (2001) as Type VI which is adapted for entrainment and dispersal within coastal currents. The other taxa 7 and 8 fit into Smayda and Reynold's Type I classification for dinoflagellates which usually inhabit relatively shallow, nutrient enriched habitats, which can be mesohaline and have reduced water-mass exchange with offshore waters. Boston Bay is a shallow nutrient enriched environment with nutrients coming from the sewage treatment works and more recently from fish

farming activities. The presence of *K. brevis* in inshore waters is probably related to nutrient concentrations (Geesy and Tester 1993).

K. mikimotoi bloomed during the cooler months of 1997/98. It is found in Japan and blooms regularly in Tanabe Bay, during late August to September (Takeuchi *et al.* 1995). Smayda and Reynolds (2001) classify this organism as Type IV. These types are adapted to the increased velocities associated with frontal zones, to the dampened but still elevated vertical mixing during relaxations in coastal upwelling or while entrained within coastal currents *i.e.* frontal zone (Type IV). Other types that fit this category are upwelling relaxation (Type V) and coastally-entrained (Type VI) HAB assemblages. All three of these types can also survive in similar habitats and are therefore interchangeable. All three types survive dispersal and can grow whilst entrained within coastal currents and frontal zones as well as nutrient rich environments (Smayda and Reynolds 2001).

K. mikimotoi and *K. brevis* bloomed in Boston Bay at different times of the year. *K. mikimotoi* counts were always lower than counts for *K. brevis*. The distributions and bloom times of these organisms are discussed at length in a later chapter.

The diatoms *Navicula* spp. are found in cool/temperate waters (Hasle and Syvertsen 1997) and bloomed in Boston Bay and surrounds in March 1998. In the samples, *Navicula* spp. was made up of solitary benthic forms at different life stages, mainly species *N. directa*, *N. distans*, *N. transitrans* var. *derasa* and f. *delicatula.* These had been resuspended from the

bottom or detached from substrate (or fish cage nets) to appear along with other marine plankton.

Guinardia striata bloomed in January. *Dactyliosolen mediterraneus* bloomed mainly in late summer and autumn, and are common in most waters but rarely a dominant species (Hasle and Syversten 1997). These diatoms are found to co-occur during the summer months in areas of high salinity >28-38 in the Southern Adriatic, Mediterranean Sea (Jasprica and Caric 2001) and continental shelf off Parana State, south eastern Brazil (Brandini and Fernandes 1996).

Pseudonitzschia spp*, Cylindrotheca closterium, and Leptocylindricus danicus* bloomed mainly in June and *Chaetoceros* spp. bloomed from March to June. *Pseudonitzschia* spp. are chain forming diatoms, some of which are benthic and others pelagic. *Cylindrotheca closterium* is a chain forming benthic diatom that has been resuspended into the water column from sediment or substrate (*i.e.* fish cage nets) whereas *Leptocylindricus danicus* and *Chaetoceros* spp. are chain forming planktonic forms. The cyanobacterium *Trichodesmium erythaeum* bloomed from January to March. The diatom *Nitzschia longissima* bloomed significantly in March at the lower Spencer Gulf site.

It is possible that the diatoms in the area are mainly controlled by temperature given the timing of their bloom periods. This has been observed in other studies where the diatoms form the dominant organism in phytoplankton communities (Brandini and Fernandes 1996).

Dinoflagellates were significant at Boston Bay sites and the upper Spencer Gulf site particularly *Karenia brevis, K. mikimotoi, Gyrodinium* spp. and the smaller gymnoids *Katodinium* spp., *Heterocapsa* spp., and *Torodinium* spp. Studies in Denmark in the Kattegatt have shown that dinoflagellates are often important in the phytoplankton community during periods of large nanoplankton biomass, particularly larger diatoms (Hansen 1991). Diatom blooms were very low during the *Karenia brevis,* and *K. mikimotoi* blooms, which may have been due to grazing by the heterotrophic dinoflagellates (Hansen 1991) as well as the ability of dinoflagellates to out-compete diatoms when conditions are optimal. Dinoflagellate significance may be a common feature in Australian temperate bay waters.

Conclusions

This study has provided data on phytoplankton biomass, dominant species composition and abundance around the tuna cages in Boston Bay and Spencer Gulf. Results of this study indicate that despite the large loading of soluble wastes, effects on phytoplankton biomass were localised. However, this study did not address the advection of nutrients from fish farms into other areas and what effects this may have on other habitats and organisms. Chlorophyll *a* concentrations appear to have increased over time and this increase was widespread rather than localised around the tuna cages. Further investigation would determine if patches of phytoplankton persist at a distance from the cages.

Several potentially toxic phytoplankton were identified in this study. The potentially ichythotoxic unarmoured dinoflagellates *Karenia brevis* and *K. mikimotoi* were dominant during 1997/98. Therefore their distribution should be investigated further to determine if they begin their bloom cycle in Boston Bay and then advect to other sites or if they are advected into the bay from off-shore areas. Information on the environmental ranges of these organisms is needed to predict the consequences of increased nutrient loadings in South Australian marine waters.

Chapter 3

Karenia brevis and *K. mikimotoi* 'look-alikes' distribution and abundance around tuna farms in Boston Bay, Port Lincoln and near-shore Spencer Gulf during 1997/98 bloom

Introduction

Harmful algal blooms (HABs) have increasingly become a problem worldwide both economically and socially. Investigations of HABs are therefore important. Approximately 40 species of phytoplankton are known to be toxic to humans and marine organisms (Hallegraeff 1995; Anderson *et al.* 2005). HABs are responsible for severe economic damage to the aquaculture industry worldwide. Several potentially toxic phytoplankton have been identified in samples from Boston Bay and Spencer Gulf in South Australia. HAB organisms prominent at the time of this study were the potentially toxic dinoflagellates *Karenia mikimotoi* (formerly *Gymnodinium mikimotoi* and *G. nagasakiense*) and *Karenia brevis* (formerly *Gymnodinium breve* and *Ptychodiscus brevis*) 'look-alikes'. The term 'look-alikes' is used, as they were identified with light micrographs and live samples and not electron microscopy.

Karenia brevis

K. brevis produces a brevetoxin which is responsible for neurotoxic shellfish poisoning in shellfish and humans. A variety of phytoplankton-related toxins have been associated with blooms of these organisms (Kirkpatrick *et al.* 2004). The most important group of brevetoxins produced is known as PbTx-2 as well as lesser amounts of PbTx-1 and

PbTx-3 (Baden 1989; Pierce *et al.* 1992). These toxins are believed to affect sodium transport in the autonomic nervous system and cause inhibition of neuromuscular transmission in skeletal muscle (Baden 1983; Purkerson *et al.* 1999). Many species of fish are sensitive to brevetoxins and effectively drown in red tide waters, as the toxin causes paralysis of the gills. Reports link *K. brevis* to the death of manatees, dolphins, clams, wild fish populations and birds (Kirkpatrick *et al.* 2004). *K. brevis* has historically been limited to the west coast of Florida (Geesey and Tester 1993) but is now common on the east coast of Florida, the coast of Texas and North Carolina (Warlen *et al.* 1998). Other species producing the same or similar toxins occur throughout the world, particularly New Zealand (Chang 1998; Rhodes *et al.* 1993; see literature review of Florida red tides by Kirkpatrick *et al.* 2004).

In Florida, blooms of *K. brevis* initiate offshore and are subsequently carried inshore by wind and current conditions where near shore samples contained greater concentrations than offshore (Geesey and Tester 1993). Blooms of *K. brevis* may be related to increased nutrient concentrations in coastal areas (Geesey and Tester 1993). In the Gulf of Mexico *K. brevis* blooms are occasionally carried around the base of Florida by the Loop Current and northward by the Gulf Stream (Tester and Fowler 1990), resulting in red tides on the east coast of Florida and, in a single incident in 1987, as far north as North Carolina.

K. brevis is motile and positively phototactic concentrating near the surface during the day (Tester and Fowler 1990) and distributing itself

generally in the water column at night or in bottom waters (Kamykowski *et al.* 1998) to access sediments as a nutrient source (Allan 2005). Migration of *K. brevis* up and down the water column may allow it to exist further offshore in regions where benthic microalgae are light limited. Populations surviving near the bottom may grow undetected in offshore and oligotrophic waters where non-motile phytoplankton would not be able to exploit near-bottom nutrients (Allan 2005). *K. brevis* moves up or down the water column at a rate of approximately one m/h (in Allan 2005). *K. brevis* has a high salinity requirement >24 and has been recorded in bloom proportions from October to November off the North Carolina coast (Tester and Fowler 1990) and from July to September in the Gulf Stream at temperatures between 22 and 24^{o}C (Tester *et al.* 1993). *Karenia brevis* has bloomed in parallel with blooms of the filamentous nitrogen-fixing, blue-green algae *Trichodesmium erythaeum* (Paerl and Bebout 1988).

Karenia mikimotoi

K. mikimotoi was referred to as *Gyrodinium aureolum* Hulbert in the literature since its first occurrence in Norweigian waters in 1996 but is morphologically closer to the Japanese bloom forming species *Gymnodinium nagasakiense* than to the original *G. aureolum* species (Partensky *et al.* 1988).

K. mikimotoi is an ichthyotoxic unarmoured dinoflagellate, produces both haemolytic and ichthyotoxins and is responsible for numerous kills of farmed and wild fish in Australia, Northern Europe, Japan and New Zealand (Partensky *et al.* 1988; Takeuchi *et al.* 1995; Hallegraeff 1995;

Silke *et al.* 2005). *K. mikimotoi* was responsible for the mortality of farmed yellowtail, *Seriola quinqueradiata*, and other fish species during blooms in the Seto Inland Sea of Japan between 1972 and 1991 with losses exceeding $161 million in value (Imai *et al.* 1992). In West Ireland *K. mikimotoi* is associated with mortalities of wild fish and other marine organisms including flounder, echinoderms, sole, plaice, rocky shore fauna and caged rainbow trout (Jenkinson and Connors 1979; Silke *et al.* 2005). *K. mikimotoi* has been found in ammonium rich waters in Gulf St. Lawrence in Canada (Blasco *et al.* 1996), Norwegian coastal waters (Dahl and Tangen 1990) and in South Australia off the southern coastline of Eyre Peninsula (SA Water pers. comm. 1996) and has been associated with frontal systems near Sherkin Island (Raine *et al.* 1990; Raine *et al.* 2001).

Dinoflagellates are generally known to be poor competitors in phytoplankton communities and have particularly low growth responses under nitrogen limitation (Cullen and Horrigan 1981). *K. mikimotoi* growth rates are recorded at 0.32–0.47 divisions per day (Honjo *et al.* 1990; Honjo 1994). *K. mikimotoi* is able to compete more successfully in a well mixed environment (Heinig and Campbell 1992) with high nutrient recycling activity (Blasco *et al.* 1996). In places where strong winds and currents inject nutrients, mainly nitrogen, into bottom waters they are able to utilise these due to their swimming capabilities (Kamykowski *et al.* 1998). Swimming of motile dinoflagellates up and down the water column is very important in the development of blooms (Honjo *et al.* 1990). Swim

rates of *K. mikimotoi* moving down the water column have been recorded at 1.3–2.2 m/h (Honjo *et al.* 1990).

Distribution of cells and subsequent development of blooms may occur through transport in estuarine fronts (Blasco *et al.* 1996) and upwelling fronts (Raine *et al.* 1990) or develop at inshore coastal waters (Dahl and Tangen 1990) or by over wintering in estuaries and bays with the residence time of bay water regulating stock sizes (Honjo *et al.* 1990). *K. mikimotoi* is recorded as blooming bimodally during spring and autumn in Omura Bay, Japan with low salinities and a temperature range of 16–22 ^{o}C (Honjo 1994) and seasonally in spring/summer months in Gulf St. Lawrence, Canada at salinities ranging from 12–35 and temperatures ranging from 9–21^{o}C (Blasco *et al.* 1996).

Aims

Boston Bay, South Australia is the site of the largest tuna farming industry in the state. It is also extensively used for other types of aquaculture such as mussel growing and land-based abalone farming. Given the multiple uses of Boston Bay and nearby Spencer Gulf region, there is clearly a requirement for elucidation of the potential for harmful algal blooms in the area each year. This study follows the distribution and abundance of two potentially toxic phytoplankton *K. mikimotoi* and *K. brevis*.

Methods

In order to measure long term spatial and temporal trends in water quality at tuna farms and away from them, water samples were taken 6–8 weekly from 26 sites, including 10 tuna cages (TC), in Boston Bay (BB) and Spencer Gulf (SG), South Australia (Figure 1) over a period of 24 months from September 1997 to March 1999. Areas considered most unaffected by tuna farming and anthropogenic influences were: the northern most part of the bay, sites 10 and 10a; the outer eastern side of Boston Island, site 6; east and west of Rabbit Island, site 2 and 2a respectively; and Taylors Island, sites 3, 4 and 5. Site 1 was a tuna cage site positioned north of Rabbit Island in Spencer Gulf to show primarily the effect of a tuna farm in the Spencer Gulf area. Tuna cages were selected from the eastern and western side of Boston Island to show the effect of location, as currents are much slower inside the island than on the outside. Sites in Proper Bay, where much of the anthropogenic inputs occur, were also sampled primarily to compare differences in biomass, hypothesising that there may be a larger effect in this area.

Sampling of some sites was haphazard over time. All sites, including the Spencer Gulf sites are classified as near-shore environments as samples were taken within 500 m of the shoreline. Water samples were collected with a Niskin Bottle from a depth of 1 m, 7–9 m and 1 m above the seabed. Due to the well mixed nature of the bay, samples were pooled and sub-samples were then poured into one-litre bottles, half of which had been earlier prepared with 5 mL of acidified Lugol's iodine solution.

Figure 1: Map of Boston Bay (BB) and Spencer Gulf (SG), South Australia, showing all sampling sites including tuna cages (TC) and controls (C); and areas used in the statistical analysis. Area 1=west Boston Island, area 2=Proper Bay, area 3=east Boston Island, area 4=upper Spencer Gulf and area 5=lower Spencer Gulf.

Counts of both organisms began in summer of 1997 using the Sedgewick Rafter cell technique; however, both the sampling technique and counting method were changed late in 1997; therefore, this earlier data was not used here. Comparisons are made with SA Water data taken at the same time.

Replicate phytoplankton counts were enumerated using the Utermöhl method (Lund *et al.* 1958). Live organisms and light micrographs were used for identification and confirmed by Professor Gustaaf Hallegraaf, University of Tasmania. A sample of cells were concentrated and sent to New Zealand to determine brevetoxin content. Physico-chemical parameters of temperature, salinity, dissolved oxygen and pH were recorded simultaneously using a Hydrolab, DataSonde® 3 Multiprobe Logger. Visibility was measured using a Secchi disk.

Results

Description of Karenia brevis and K. mikimotoi in Boston Bay and near-shore Spencer Gulf

Karenia brevis `look-a-like'

In August 1996 a *K. brevis* `like' organism was first observed in Boston Bay water samples (SA Water unpublished data) (Figure 2). The cell was a butterfly shape, slow moving, mostly <50 μm wide and 15–30 μm in length. It had a large, apical process. Apical groove was not visible in the micrographs. This cell was dorso-ventrally flattened, concave ventrally, cingulum slightly premedian and the nucleus was round, and situated in

the left lobe of the hypotheca. The hypotheca was bilobed, and chloroplasts were present. This organism resembles one of the 3 types found in New Zealand's waters in 1994 (Haywood *et al.* 1996) and described by Steidinger and Tangen (1996).

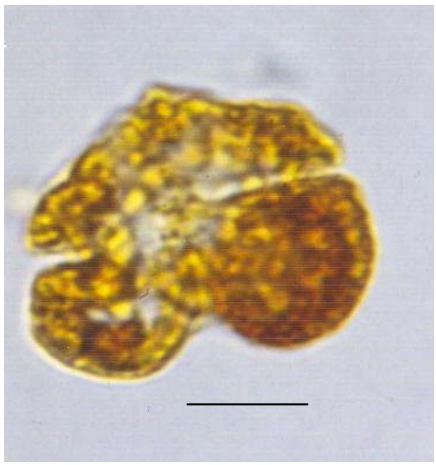


Figure 2: *Karenia brevis* 'look-alike' (scale 15µm).

***Karenia mikimotoi* 'look-a-like'**

K. mikimotoi `like' organism was first observed in samples in January 1997 (SA Water and Paxinos unpublished data) (Figure 3). It was first thought to be *Gymnodinium pulchellum* but later identification found it to be more similar to *K. mikimotoi* (SA Water pers. comm. 1997). This cell was small, yellowish brown, 25–50 µm in length and moved in a cork-screw fashion. It was flat dorso-ventrally and had a large nucleus in the left lobe of the hypotheca. A type was also found with the nucleus situated in the epitheca although this was rare. The hypotheca is larger than the epitheca, the cingulum is slightly premedian and the hypotheca bilobed.

The epitheca in most was rounded and others slightly sub-conical. These morphological features typically describe *K. mikimotoi,* particularly the shape, placement of the nucleus and cingulum arrangement as described in Steidinger and Tangen (1996). The apical groove was not visible in the micrographs.

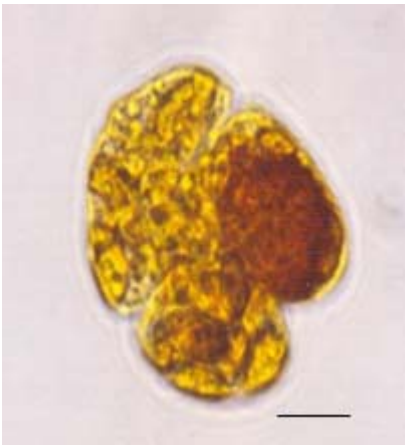


Figure 3: *Karenia mikimotoi* 'look-alike' (scale 10µm).

General distribution

Overall water column samples showed (Figure 4) that *K. brevis* production peaked in late summer and autumn of 1997–98, with the highest recorded numbers found at sites near the entrance to Proper Bay and coinciding with a large *Trichodesmium erythaeum* bloom, during January of both years. *Karenia brevis* was the dominant organism during the summer and autumn sampling periods along with *Gyrodinium* spp. and smaller Gymnoids. *K. brevis* blooms never reached what is defined as `fish killing proportions' (1–2.5x10^5 cells/L) (Tester and Steidinger 1997) in Boston Bay and there were no recorded deaths of marine organisms due to toxicity.

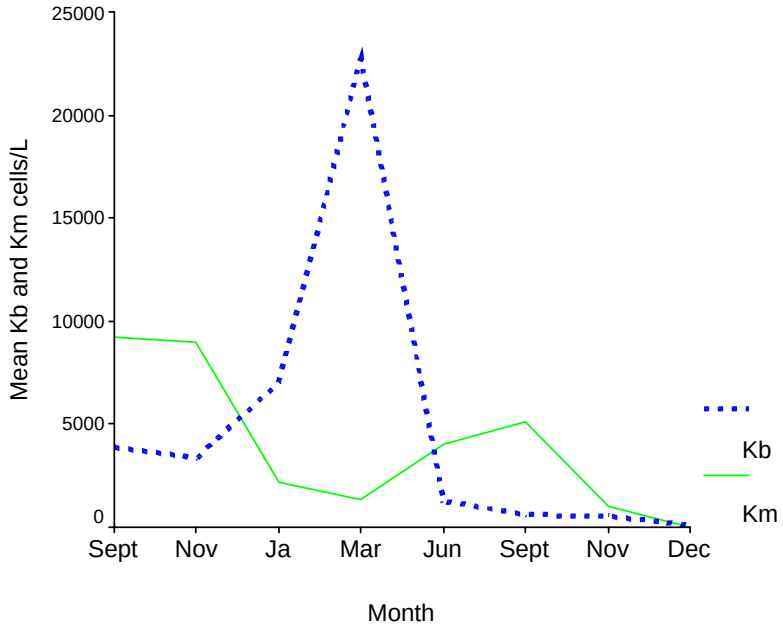


Figure 4: Mean *K. brevis* (Kb) and *K. mikimotoi* (Km) cells/L over all sites and all sampling months in 1997/98.

Very low concentrations of brevetoxin have been found in Boston Bay at different times; however, the organism responsible for the toxin has not been identified (SA Water pers. comm. 1997). *K. mikimotoi* cell counts were quite variable from site to site and cells were present in the water column in low numbers for most of the year. *K. mikimotoi* generally bloomed from May/June to September/ November in 1997/98.

K. brevis numbers were generally highest ($82.4x10^3$ cells/L) at BB15a in area 1 (Proper Bay and Spalding Cove) (Figure 5). On the western side of Boston Bay highest cell numbers ($29.6x10^3$ cells/L) were recorded at BB14 in area 2, and on the eastern side of Boston Island ($45.4x10^3$ cells/L) at site BB6a in area 3 during March/April 1998.

In 1997 data from SA Water showed that *K. brevis* mean cell numbers were highest, $52.0x10^3$ cells/L, during March/April in Boston Bay (SA Water unpublished data). In the upper Spencer Gulf (area 4) samples, *K. brevis* numbers ranged from $5.24x10^2$ cells/L to $28.0x10^2$ cells/L. Water samples from the lower Spencer Gulf sites, area 5, (Figure 5) contained no *K. brevis* or no cells in detectable numbers except during March 1998 where cells numbers were $7.14x10^2$ cells/L. They began to disappear in Sept/Nov 1998 and did not appear in such numbers during 1999 or 2000 (SA Water unpublished data).

Mean cell counts for *K. mikimotoi* at SG1/TC in upper Spencer Gulf sites, area 4 (Figure 5), increased in April 1998 from $<1.4x10^2$ cells/L to $14.9x10^3$ cells/L and decreased to undetectable levels in November. *K. mikimotoi* numbers were highest ($45.2x10^3$ cells/L) at BB12 in area 2 (western side of Boston Bay) in Sept/Nov 1997 and did not reach these numbers in 1998. *K. mikimotoi* cell counts from the lower Spencer Gulf sites (area 5) contained few or cells in undetectable numbers except during November 1997 where cells were detected at $2.5x10^2$ cells/L, in March 1998 $1.4x10^3$ cells/L and June 1998 at $1.9x10^2$ cells/L.

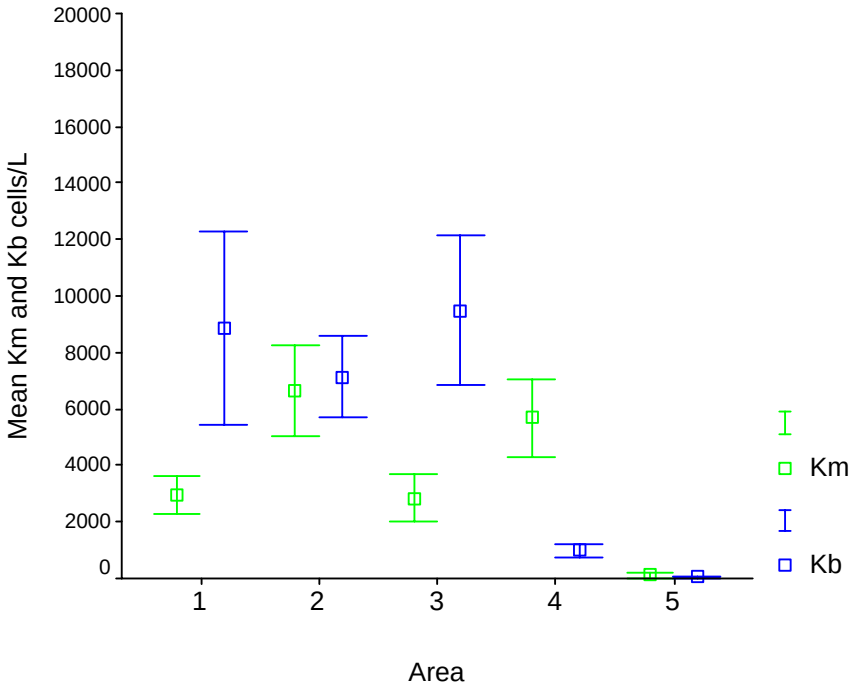


Figure 5: Mean *K. brevis* (Kb) and *K. mikimotoi* (Km) cells/L (± 1SE) by areas, (area 1= Proper Bay and Spalding Cove, area 2=west of Boston Island, area 3=east of Boston Island, area 4=upper Spencer Gulf and area 5= lower Spencer Gulf).

Figure 6 shows that during 1998 *K. brevis* cell counts increased with increasing temperatures; however, the opposite effect was recorded for *K. mikimotoi* which was dominant in 1997 but not in 1998. Low numbers of *K. mikimotoi* cells were still found in Boston Bay in 2000 (SA Water unpublished data 2000). No toxicity has been recorded for this organism in Boston Bay.

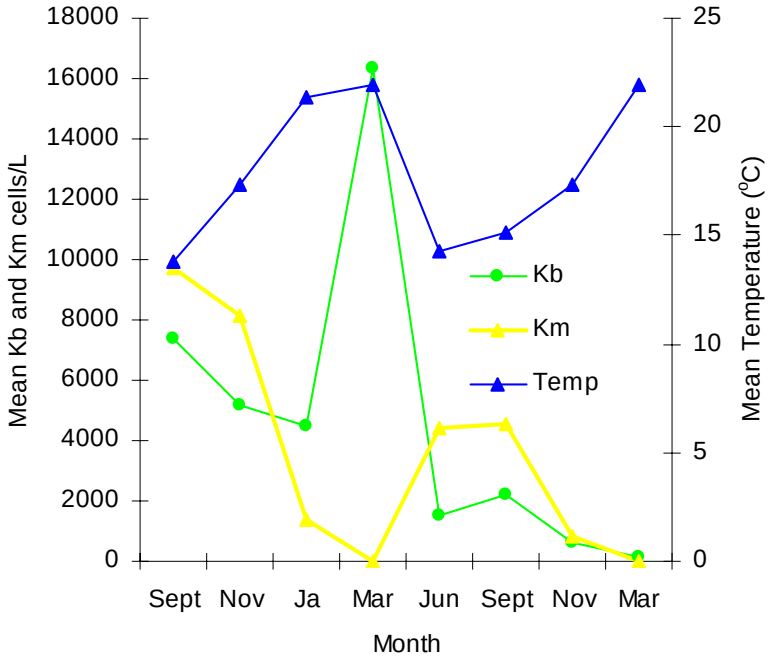


Figure 6: Mean temperatures (^{o}C) and mean cells/L for *K. brevis* (Kb) and *K. mikimotoi* (Km) over all sites and sampling months.

Distribution of *K. brevis* at tuna cages and controls

Figure 7 shows that the mean cell counts for *K. brevis* in Areas 1 to 5 at tuna cages and controls during the sampling period were higher at the control sites in areas 1 and 2 overall. Across the sampling months mean cell counts of *K. brevis* (Figure 8) were higher at tuna cages and controls during the months of January, March and June; however, these were not significantly different from each other (t-test, p=0.586).

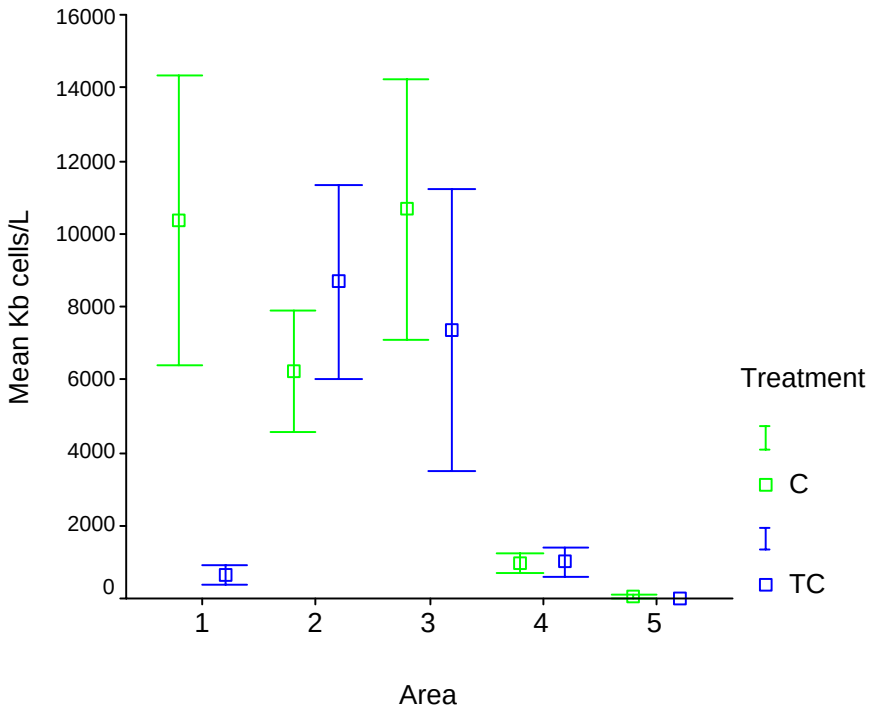


Figure 7: Mean *K. brevis* (Kb) cells/L by areas, (area 1= Proper Bay and Spalding Cove, area 2=west of Boston Island, area 3=east of Boston Island, area 4=upper Spencer Gulf and area 5= lower Spencer Gulf) at tuna cages (TC) and controls (C) (± 1SE).

K. brevis cells were quite evenly distributed across all sites on the eastern and western sides of Boston Island and upper Spencer Gulf; distribution was patchy at sites in southern Boston Bay, Spalding Cove and Proper Bay, (but cells were found deep into both Spalding Cove, site BB18 and Proper Bay, site BB15d) and were virtually undetectable in lower Spencer Gulf.

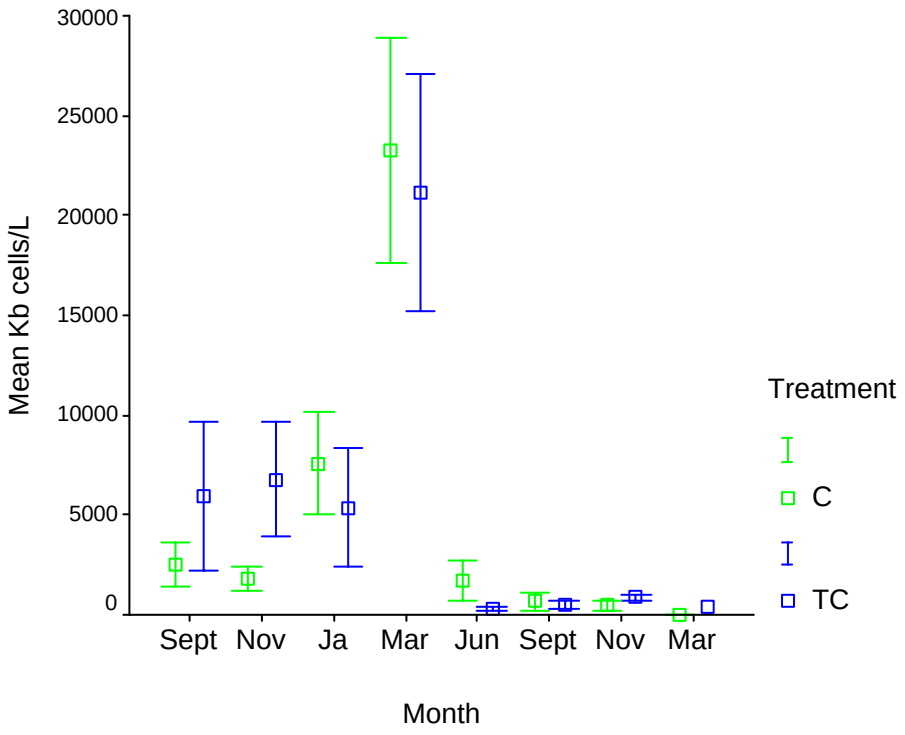


Figure 8: Mean *K. brevis* (Kb) cells/L (± 1SE) for tuna cages (TC) and controls (C) over all sites and sampling months.

Distribution of *K. mikimotoi* at tuna cages and controls

Figure 9 shows *K. mikimotoi* numbers were dominant at tuna cages in Areas 2, 3, and 4, Boston Bay, east of Boston Island and upper Spencer Gulf. Across the sampling months, mean cell counts of *K. mikimotoi* (Figure 10) were also higher at tuna cages and controls; t-tests showed that this result was significant (p=0.03). *K. mikimotoi* cells were evenly distributed across all sites on the western side of Boston Island, upper Spencer Gulf, Proper Bay and Spalding Cove (although few cells were found deep into Proper Bay, at sites BB15c and d); distribution was patchy at sites on the eastern side of Boston Island with the largest numbers of cells being recorded at sites nearest the island which were the tuna cage

sites; *K. mikimotoi* cells were virtually undetectable in lower Spencer Gulf samples.

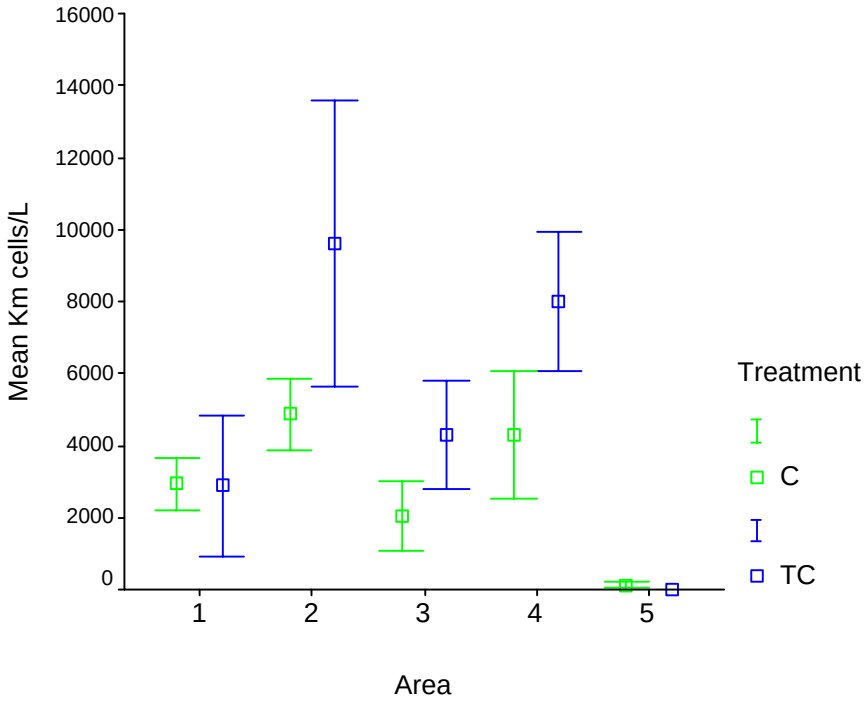


Figure 9: *K. mikimotoi* (Km) mean cells/L (± 1SE) by areas, (area 1= Proper Bay and Spalding Cove, area 2=west of Boston Island, area 3=east of Boston Island, area 4=upper Spencer Gulf and area 5= lower Spencer Gulf) at tuna cages (TC) and controls (C).

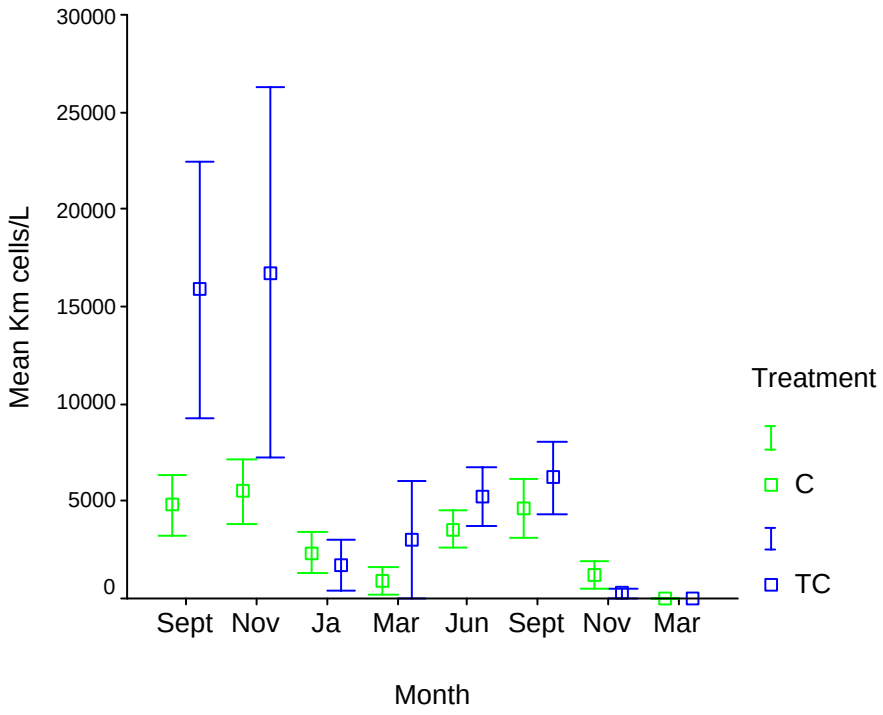


Figure 10: Mean *K. mikimotoi* (Km) cells/L (± 1SE) for tuna cages (TC) and controls (C) over all sites and sampling months.

Ammonia concentrations ranged between 5.0 and 245 μg/L at tuna cages (TC) and 5.0 and 112.0 μg/L at controls (C); however, these were not statistically significant (p>0.05). Ammonia at all stations followed a seasonal pattern that was opposite that of DO. There was a general increase in ammonia overall from January to March in 1997/98. *K. brevis* cell counts also rose during the months of autumn and followed the ammonia trend over the sampling period. *K. mikimotoi* cell counts decreased as ammonia levels increased (Figure 11).

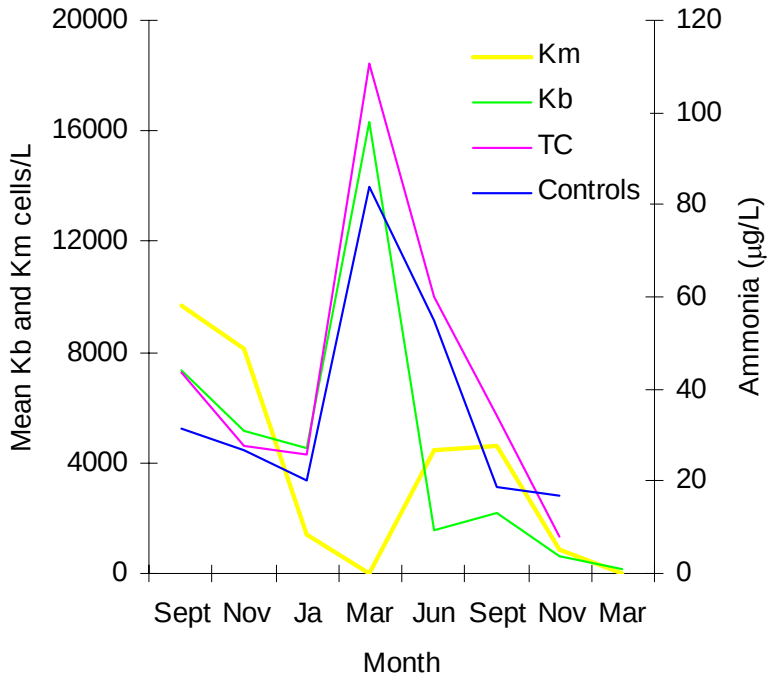


Figure 11: Mean ammonia (µg/L) levels at tuna cages (TC) and controls (C) compared to mean *K. brevis* (Kb) and *K. mikimotoi* (Km) cells/L for all sites and sampling months.

Temperature followed the same seasonal pattern at tuna cages and controls and there was little variation between surface and bottom temperatures (see Chapter 2). The mean average temperature was at a maximum in March (21.7^{o}C) and minimum in September (13.5^{o}C). *K. mikimotoi* cell counts decreased as temperature levels increased and *K. brevis* cell counts appeared to increase only when temperature was at its maximum in autumn (Figure 12).

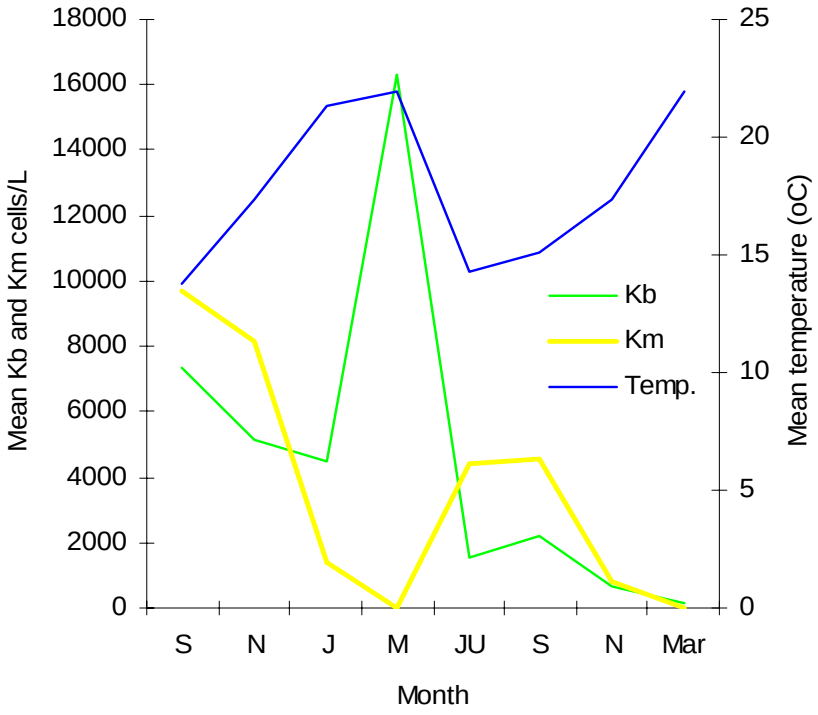


Figure 12: Mean water temperature (^{o}C) compared to mean *K. brevis* (Kb) and *K. mikimotoi* (Km) cells/L for all sites, over all sampling months.

Discussion

Karenia brevis

K. brevis cells were widely dispersed in Boston Bay and did not appear to be associated with any particular fish farm site. This is probably due to *K. brevis* motility. Cells undergo a diel vertical migration where they aggregate at the surface during the day and are able to disperse widely in the water column during the night (McKay *et al.* 2003). *K. brevis* bloomed in summer and autumn in large numbers in Boston Bay (see Figure 1) and cells appeared in lower Spencer Gulf samples in very low numbers in March 1998. Numbers were also low at the upper Spencer Gulf sites. Tester *et al.* (1993) found that variations in the distribution and abundance of *K. brevis* have been linked to changes in water temperatures and circulation as has been found in Florida waters where cells are transported by the Gulf Loop current. Water temperatures were no different at the upper Spencer Gulf sites from sites in Boston Bay; therefore, the cells found at these sites were more likely transported from the Bay through the northern channel and into the Gulf.

Geesey and Tester (1993) hypothesised that the greater abundance of *K. brevis* in near-shore waters in Florida may be related to increased nutrient concentrations; however, other reports suggest that *K. brevis* appear to be unaffected by coastal nutrient enrichments (Hallegraeff 1995). Nitrogen and phosphorous were not considered to play a role in the initiation or sustenance of *K. brevis* blooms. Studies in coastal Florida waters found no correlation between red tide occurrences and nitrogen or

phosphorous (Geesey and Tester 1993). However, experiments have shown that *K. brevis* cells grow quite successfully on different types of nitrogen, particularly nitrate, ammonium and urea (Richardson and Pinckney 2003). Ammonia was particularly prevalent in Boston Bay during the time of the *K. brevis* blooms.

Blooms of *K. brevis* have also been associated with blooms of the filamentous nitrogen fixing cyanobacterium *Trichodesmium erythaeum.* In January 1997 and 1998 unusually calm, sunny and clear weather was observed for several weeks in Spencer Gulf. This led to a large bloom of the filamentous nitrogen fixing cyanobacterium, *Trichodesmium erythaeum.* Aerial photographs confirm the bloom extended for over 20 nm from south of Taylor's Island north to the Sir Joseph Banks Group of islands. Blooms of *T. erythaeum* have been associated with the parallel development of *K. brevis* blooms (Paerl and Bebout 1988; Tester *et al.* 1993). It is possible that the presence of *K. brevis* in Boston Bay waters during this time was associated with the cyanobacterium bloom. Mulholland *et al.* (2003) demonstrated in field and laboratory experiments that N released from *T. erythaeum* can support the growth of *K. brevis.* Results also showed that *T. erythaeum* can fix N_2 at high rates with more than 50% of this new N released as NH^{+4} and DON, and that *K. brevis* has a high affinity for reduced N sources. *K. brevis* blooms were most likely influenced by water temperatures input of nitrogen from the *T. erythaeum* bloom and also ammonia excreted from fish in the tuna farms.

Other studies have linked occurrences of red tides with large fluvial inputs of iron. Dissolved Fe concentrations in a Florida estuarine red tide were significantly lower than in a nearby diatom bloom, suggesting a greater Fe requirement by the red tide dinoflagellate (Geesey and Tester 1993). The bloom potential of *K. brevis* may be controlled in part by such trace metals, commonly delivered to coastal waters through river run-off. Recent studies have demonstrated that although humic substances are taken up by *K. brevis*, they are not a significant source of N to *K. brevis* blooms (Heil 2003).

Karenia mikimotoi

In 1998, on the western side of Boston Island (area 2), cell counts of *K. mikimotoi* were on average $<12.0 \times 10^3$ cells/L compared to 1997 where they peaked at averages of $>20.0 \times 10^3$ cells/L in September/November samples. In January 1998, numbers of *K. mikimotoi* were 7.7×10^3 cells/L in area 2 and 1.1×10^3 cells/L in area 1 but $<2.0 \times 10^2$ cells/L in area 3, 4 and 5. In March, numbers in area 4 increased to $>9.3 \times 10^2$ cells/L but were $<3.0 \times 10^2$ cells/L in all other areas. In June *K. mikimotoi* increased its numbers in area 1 and 2 and then in September in area 3 and 4. The lag time for *K. mikimotoi* appearing at sites east of Boston Island and upper Spencer Gulf leads to the assumption that *K. mikimotoi* cells on the western side of Boston Island were advected to upper Spencer Gulf sites by the currents that move water out of Boston Bay through the northern channel. Takeuchi *et al.* (1995) note that the movement of *K. mikimotoi* blooms in Tanabe Bay, Japan, from the southern area to the northern area

and then toward the entrance of the bay during the last stage of the bloom, was consistent with a clear counterclockwise current in the bay and implied that the distribution of *K. mikimotoi* blooms is at least partly dependent upon this current.

Cell numbers for *K. mikimotoi* in Boston Bay and Spencer Gulf, even at their highest recorded numbers of $52.0x10^3$ cells/L, were very low compared to major blooms in other countries. For example, in Omura Bay, Japan, cell numbers during a major *K. mikimotoi* (formerly *Gymnodinium nagasakiense)* bloom exceeded $1.0x10^6$ cells/L (Ishimaru *et al.* 1989). Therefore it would appear that the growth of *K. mikimotoi* was somewhat limited in Boston Bay and surrounds.

K. mikimotoi has a slow growth rate at 0.32–0.47 divisions/day (Honjo *et al.* 1990; Honjo 1994). Honjo *et al.* (1990) suggest that low rates may be the result of loss of cells to other areas by advection, and may result in a long interval between blooms appearing. Low growth rates in *K. mikimotoi* are also exacerbated by nitrogen limitation. Ammonia levels were high in Boston Bay during 1998; therefore, it is suggested that *K. mikimotoi* was not nitrogen limited.

K. mikimotoi swims up and down the water column with swim rates recorded down the water column at 1.3–2.2 m/h (Honjo *et al.* 1990), which enables it to utilise nitrogen from bottom sediments. Depths at sampling sites during this study ranged from 13–22 m. At these depths, it would take *K. mikimotoi* approximately 16–30 hours to swim up and down the

water column. During this time *K. mikimotoi* cells could be advected to other areas by surface and bottom currents operating in the bay, depending on the speed of the currents. Advection of cells may be slow, as current speed rates in the bay are 0.02–0.04m/s (Noye 1996), which may account for the cell numbers being larger in the bay than anywhere else. Motile dinoflagellates are able to concentrate at comfortable depths due to their motility even under turbulent or upwelling conditions (Yamamoto and Okai 2000).

Flow is up and outwards through the north channel and there is a weak clockwise flow around Boston Island. At low water at Port Lincoln during a neap tide, current speeds are very small or non-existent, this may last for around 3 hours. Noye (1996) suggested when the tide turns in Boston Bay the water at Port Lincoln is virtually stationary and currents in Boston Bay and Proper Bay are almost negligible but currents remain large in Spencer Gulf. This indicates that pollutants or organisms located on the western side of Boston Island will tend to accumulate in Boston Bay. *K. mikimotoi* cells would be likely then to accumulate in the bay and, given the weak clockwise flow around the island, this may account for the accumulation of *K. mikimotoi* cells east and west of Boston Island around the tuna cages at the sites nearest the island.

Temperature may not be a limiting factor as *K. mikimotoi* growth rate is generally independent of rising or falling temperatures (Honjo *et al.* 1990). However, studies have shown that cells of developing populations have a requirement for mixing by wind, reduced salinity by rainfall or other

freshwater inputs (Honjo *et al.* 1990) as well as a requirement for the trace metal selenium (Ishimaru *et al.* 1989). Boston Bay is well mixed and wind is common, particularly in the afternoons from the south-west. Salinity may be a limiting factor. A study in Canada determined that salinities for *K. mikimotoi* range from 12–35 (Blasco *et al.* 1996) with 35 being at the extreme end of the range. The highest growth rates of *K. mikimotoi* observed in laboratory experiments were at the combination of temperature at 25^{o}C and salinity at 25 (Yamaguchi and Honjo 1989). The range of salinities that sustained growth at 0.5 divisions/day was relatively wide (15 to 30) (Yamaguchi and Honjo 1989). Salinities in Boston Bay and surrounds are generally stable at approximately 36–37; therefore, salinity may be a limiting factor in the growth of this organism during this study. It is not known if the trace metal selenium occurs in Boston Bay and surrounds.

Motile cells of *K. mikimotoi* and *K. brevis* are still present in Boston Bay and Proper Bay in very low numbers (SASQAP 2006) although they do not appear to have achieved the growth rates evident in this study. These organisms obviously have mechanisms for maintaining themselves in the water column despite suboptimal conditions. In Gokasho Bay, Japan, most *K. mikimotoi* cells have been observed during the day at 5–10 m, which suggests that maintaining themselves in the middle layer may be important in avoiding the frequently unfavorable conditions encountered at or near the surface (Honjo *et al.* 1990). Persistent low levels of *K. brevis* cells have been recorded over-wintering in Florida waters and blooms are associated with temperatures between 22–24^{o}C (Tester *et al.* 1993), and

temperatures between 5–10^{o}C appear to inhibit its growth (Tester *et al.* 1993).

Sites in the middle of Spencer Gulf have not been sampled for *K. brevis* or *K. mikimotoi* cells or further north from Rabbit Island sites (SG1) so cells may occur in these areas but no blooms are recorded. A northward flowing current enters the Spencer Gulf from the Bight region, travelling along the westerly entrance of the gulf. The northerly current continues to flow up the gulf until deflected at Tiparra Reef. This deflection creates a counter-current that runs along the opposing shore, which merges with waters from east to west along the entrance of the Spencer Gulf (Bullock 1975; Bye and Whitehead 1975). Therefore, there is the opportunity for dispersal of these organisms into other areas and continual inoculation of Boston Bay sites with over-wintering cells.

In conclusion, this study follows the distribution and abundance of two potentially toxic phytoplankton *K. mikimotoi* and *K. brevis* in Boston Bay, Port Lincoln. The blooms of both of these organisms appeared to begin in the bay and were then advected to other areas by the prevailing currents. These organisms did not bloom in fish killing proportions because it appears that the growth of both *K. mikimotoi* and *K. brevis* was somewhat limited in Boston Bay and surrounds. From this study, it is unclear what these growth limiting factors were. Future studies may shed some light on the growth limiting factors of both of these organisms in the South Australian marine environment.

Chapter 4

Phytoplankton *in vivo* fluorescence and size fractionated chlorophyll *a* measurements along directional transects around tuna farms in Boston Bay, Port Lincoln

Introduction

Localised eutrophication is often reported from fish farm sites (Rensel 1989; 1990; Wu *et al.* 1994; Troell and Berg 1997; Arulampalam *et al.* 1998; Clarke 1996; Pitta *et al*. 1999; CSIRO 2000). Fish farming practices in the marine environment enrich the surrounding water column through the addition of faeces, urea, uneaten food, desquamation, mucus, vitamins and therapeutic agents (Wildish *et al.* 1990), the accumulation of which is exacerbated by low water movement that characterises some locations (Braaten *et al*. 1983, Tsutsumi 1995). Comprehensive changes in water quality are caused by elevated ammonium, nitrate and nitrite levels (Braaten 1983; Weston 1986; Gowen and Bradbury 1987; Kaspar *et al.* 1988; Caine 1989; Lumb 1989; Rensel 1989, 1990; Hall *et al*. 1992); hydrogen sulphide outgassing in sediments (Braaten *et al*. 1983; Lumb 1989; Bagarino and Lantin-Olaguer 1998) and elevated phosphorous levels (Foy and Rosell 1991; Holby and Hall 1991). Degradation of water quality around fish farms has become severe enough in places to cause mortalities of farmed fish and other marine organisms, through oxygen starvation or toxic poisoning (Weston 1986; Honjo 1994).

The release of dissolved nutrients from fish excreta and resuspension of nutrients from sediments from beneath fish farm cages can increase

phytoplankton growth (Pease 1977; Weston 1986; Caine 1989; Rensel 1989, 1990; Troell and Berg 1997). The resulting increase in phytoplankton biomass may provide an advantage to toxic dinoflagellates (Smayda 1990). Therefore, detection of phytoplankton blooms in the marine environment is required, not only to describe the frequency and variation in chlorophyll accurately, but also for the protection of aquaculture sites and for implementation of measures to protect public health (Cullen *et al*. 1997).

Studies around coastal marine fish farms have given somewhat complex and variable results, which, according to reports, make it difficult to associate a direct link between fish farm hyper-nutrification and phytoplankton growth (Troell *et al.* 1999). Despite studies indicating that at certain times of the year around fish farms phytoplankton biomass increases, this is often difficult to quantify because of the patchy nature of phytoplankton in the marine environment. Patches form when biological processes such as reproduction, growth, grazing and predation become larger than the physical processes of turbulence, diffusion and advection (Cebrian and Valiela 1999). The term patchiness is generally used to describe biological or physical variability at horizontal scales between about 10 m and 100 km and at vertical scales between about 0.1 m and 50 m. (Mackas *et al*. 1985).

Phytoplankton patchiness is complicated by biological and physical variability, which will alter the number, timing and magnitude of annual blooms, remarkably, among close locations (Patten *et al*. 1963 Stockner *et*

al. 1977) and among years within the same location (Motoda *et al*. 1987; Uhlig and Sahling 1990; Harris *et al*. 1991). Further complication arises in coastal areas where water movement (turbulence) is rapid, allowing for relatively intense horizontal exchange (Tett 1986), which enables hypernutrified waters to exchange quickly with their surroundings (Gowen *et al*. 1992) or, conversely, water movement is slow, acting to maintain nutrients and organisms within a particular habitat. Turbulence is often considered the mechanism for producing phytoplankton patchiness (Waters 2003).

The ecological significance of the formation of persistent patches of phytoplankton around fish farms is identified in the long term stability of fish farms at particular sites and may be due to large soluble nutrient input, sediment build up below the fish farms and subsequent continual resuspension of nutrients over time. Very slow and unobserved eutrophication of an area is therefore probable and in the long term may well be detrimental for both social and economic reasons. This eventuality is very real when considering that most fish farming zones are never rotated to eliminate build up beneath cages, particularly in shallow coastal waters. To date there have been no studies which address the issue of patchiness around fish farm sites.

Continuous flow *in vivo* fluorescence

A dilemma often exists between the sampling scale and the ecologically relevant scale, which may result in inaccurate interpretation of phytoplankton ecology (Waters 2003). Largely this occurs when using

discrete sampling techniques, particularly at low spatial resolution. The recording of data by continuous flow *in vivo* fluorescence to quantify the patchy distribution of phytoplankton came into use in 1966 (Lorenzen 1966). This innovation has since generated vast amounts of literature, which describe the spatial and temporal variability in phytoplankton (Platt 1972; Platt and Denman 1975; Powell *et al*. 1975; Lekan & Wilson 1978; Abbott *et al.* 1982; Weber *et al.* 1986; Powell and Okubo 1994; Strutton *et al.* 1997; Abraham 1998; Seuront *et al.* 2002; Waters 2003). *In vivo* fluorescence is an extremely sensitive and specific method for describing the distribution of marine phytoplankton (Falkowski and Kolber 1995; Cullen *et al.* 1997). It is also useful because measurements can be made continually along (horizontal) transects, vertical profiles, or long deployments (Cullen *et al.* 1997). Continuous measurements also allow unambiguous interpretations that are often not possible with the more traditional methods based on sampling at discrete stations (Pingree *et al*. 1975). Therefore, rather than discrete point sampling, continuous measurements of *in vivo* fluorescence may be a useful tool in describing spatial and temporal variability in chlorophyll biomass around fish farms.

Size fractionated chlorophyll

Another method that has provided insight into phytoplankton dynamics is to measure the size structure of the biological community (Malone 1980; Jiao and Wang 1994; Bradford-Grieve *et al.* 1997; Cottingham 1999; Yew-Hoong Gin *et al*. 2000; Jacquet *et al.* 2006). Studies of size-fractionated chlorophyll have shown that for open ocean waters, approximately 60-98% of chlorophyll is contributed by the pico- (<2 μm) and nanoplankton (2–20

μm) (Berman 1975; Hopcroft and Roff 1990). In contrast, coastal environments are generally dominated by microplankton (>20 μm) due to their competitive advantage in highly fluctuating nutrient environments (Malone 1980). Studies have shown that changes in size structure are predicted following perturbations of nutrient inputs and (or) food web structure in freshwater and marine environments (Carrick and Schelske 1997; Cottingham 1999). Thus, changes in size structure can be used to reflect phytoplankton responses to perturbations in the environment, with implications for ecosystem function and health (Yew-Hoong Gin *et al*. 2000).

Aims

Boston Bay is located on the western side of Spencer Gulf in South Australia. Tuna farms are situated to the east and west of Boston Island and in Spencer Gulf to the North of Rabbit Island and Louth Bay. Previous studies in Boston Bay (Clarke 1999 and chapter 2) showed that chlorophyll biomass and nutrient levels around tuna farms were elevated at particular times of the year. However, interpretation was difficult, as the studies used discreet point sampling at 4–8 week intervals, so that much of the spatial and temporal variation was not available. This study identifies the relationship between tuna farms and phytoplankton biomass through identification of temporal and spatial structure of *in-vivo* fluorescence, and by comparing this to total chlorophyll *a* and size fractionated chlorophyll *a* taken with discreet water sampling techniques using directional transects around selected tuna farm sites.

Methods

Experimental design

Two tuna cages (TC) were selected haphazardly; these had been stocked with southern bluefin tuna in late December 1998 and early January 1999. The cages were 50 m in diameter with rubber pontoons at the surface and rope mesh anchored in the seabed. TC1 (Figure 1) was situated on the western side of Bickers Island. This farm had been in operation for four years, in the same position, and was therefore the older site of the two farms selected (Clarke *et al*. 1999). TC1 was in a farm of three cages but was not attached to the other two and it was in water approximately 16 m deep on the southern side of the south channel. TC2 (Figure 1) had been operative since December 1998 (Clarke *et al*. 1999). It was situated on the mid-western side of Boston Island, was attached by ropes to one other tuna cage and was in water approximately 18 m deep; both farms were approximately 750 m off-shore and contained fish.

The controls sites (C) (Figure 1) were chosen with two variables in mind: that they were far enough from the tuna farms so as not to be influenced by inputs/outputs of the farms themselves and were in areas where no tuna cages had previously been stationed. The control for TC1 was 2 nautical miles (nm) west of the tuna cage and for TC2 the control was 2 nm north of the tuna cage.

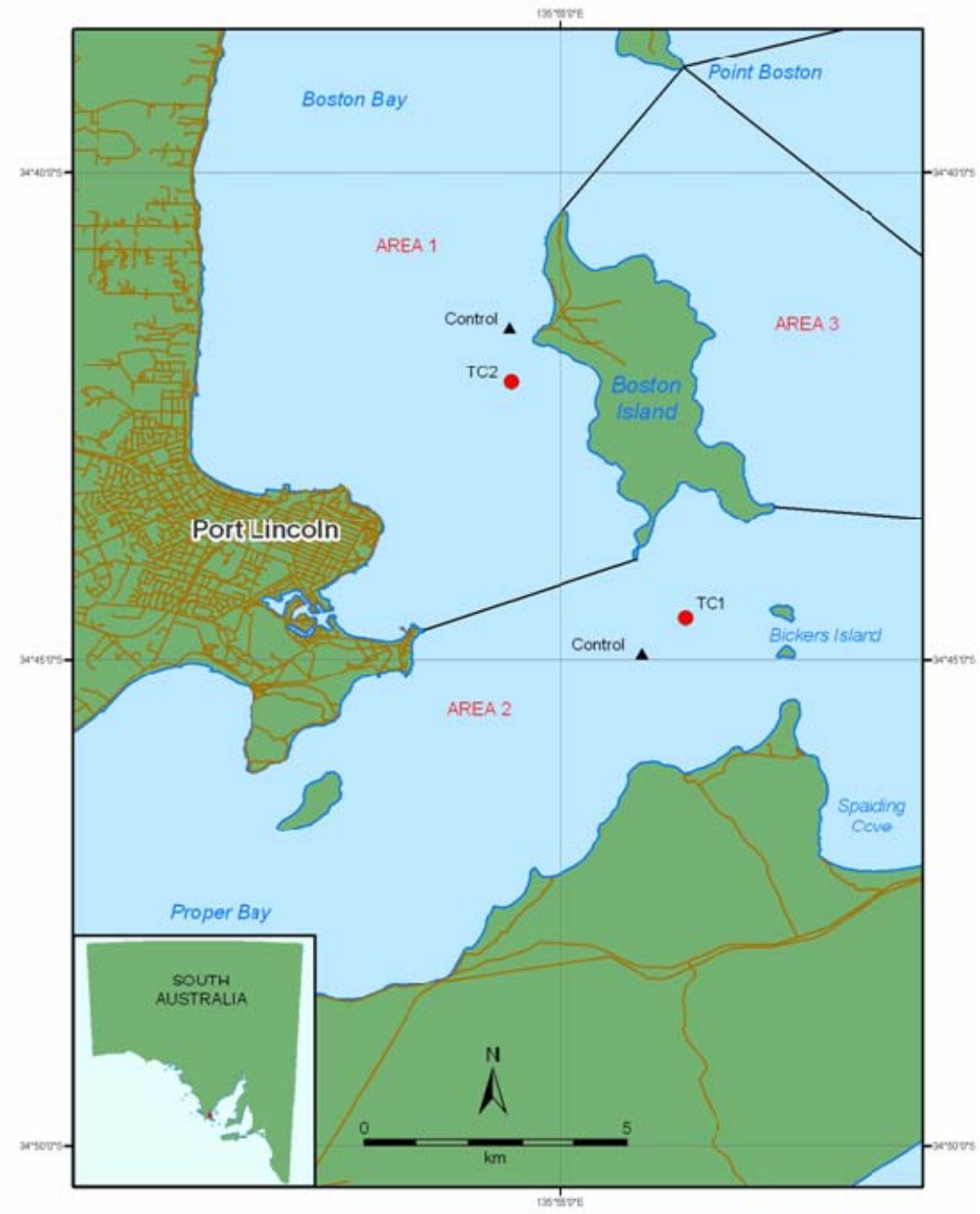


Figure 1: Map of Boston Bay, South Australia showing tuna cage 1 (TC1) and 2 (TC2), red circles, and control sites 1 (C1) and 2 (C2), black triangles.

In vivo fluorescence, chlorophyll *a* size fractionation and abundance

Three replicate transects were placed at equal distance apart around each tuna cage and control site (Figure 2): they were approximately 500 m

in length and generally faced north, south, or west. Buoys were anchored at the control sites to simulate a tuna cage and the same transects used at the tuna cage sites were then repeated at the control sites. GPS readings were taken at the beginning and end of each transect to ensure that sampling was conducted at the same transect on each sampling day.

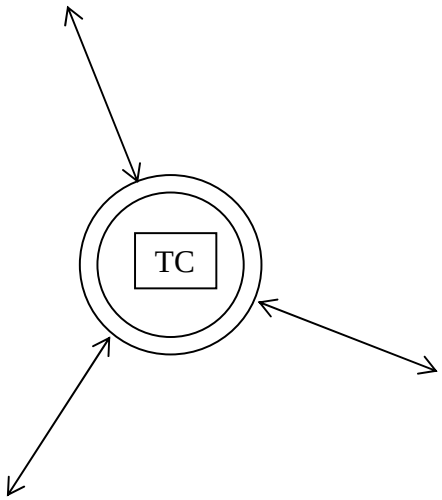
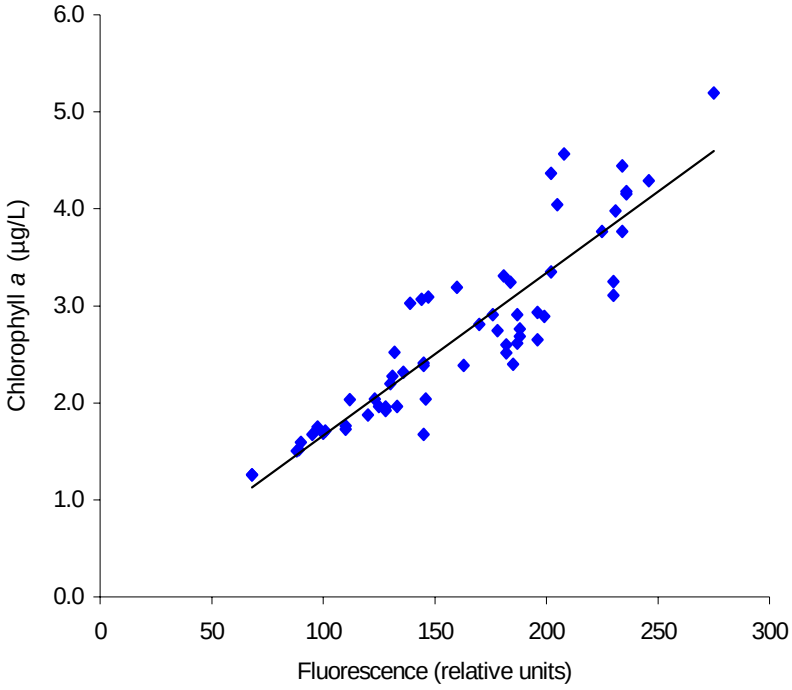


Figure 2: Diagrammatic representation of the direction of the tuna cage and control transects. Three replicate transects were placed at equal distance apart around each tuna cage and control site: they were approximately 500 m in length and generally faced north, south, or west.

Continuous surface water measurements of *in vivo* fluorescence and temperature were recorded with a Turner 10-AU Model Fluorometer, and calibrated by *in vitro* chlorophyll measurements. Figure 3 shows the calibration curve for conversion of fluorescence to chlorophyll *a* (μg/L). Measurements were taken on the incoming tide. The pump was attached to the side of the boat and at a depth approximately 1 m from the surface

of the water. Data was recorded at one second intervals, the boat was moving at a speed of ~2 knots/hour and the current was moving at approximately 0.2 m/s which meant that spatial resolution of data was



approximately 1.5 m. All samples were taken on the incoming tide.

Figure 3: Calibration curve for the conversion of fluorescence (relative units) to chlorophyll *a*, derived by chlorophyll *a* extraction of water samples taken from the outlet of the flow-through fluorometer. The calibration equation is: Chl *a* (µg/L) = 0.0168 x F-0.0138 where fluorescence is F (n=60, r^2=0.8119, correlation coefficient = 0.901, p<0.01).

Fluorescence readings rise in relation to rising temperatures (Jeffrey 1996) but as the flow through fluorometer was temperature compensated, transects were not continuous and the variation in temperature was so minimal, there was no corresponding rise in the fluorescence readings.

Known volumes of seawater were taken at 0 (cage edge), 50 and 500 m from the fluorometer's outlet and filtered through a 300 μm mesh filter. The samples were size fractionated by re-filtering onto 5 μm Milllipore GS filters and then passing the filtrate through 0.45 μm filters. Filters were immediately frozen and stored in darkness at -20°C for subsequent extraction of chlorophyll *a*. For determination of cell abundance and identification, one litre whole water samples taken from the outflow of the fluorometer were preserved in 5 mL Lugol's iodine solution. Replicate samples were enumerated using Utermöhl's settling technique (Lund *et al*. 1958). The study took place over a period of 15 days. Transect recordings and samples were taken on every third day starting from day one. During this period tidal streaming moved from spring tide on the first two sampling days slackening towards neaps on days 3 and 4 and back to spring tide on day five. Salinity and temperature were recorded using a Hydrolab, DataSonde® 3 Multiprobe Logger.

Data analysis

We performed statistical analysis of the raw data using a Multivariate Analysis approach. Analysis of the transect data was done by observing the time plots and using spectral analysis. SPSS 9.0 statistical program was used for the data analysis; all analyses are explained in text. Transect data was converted so that all data was running in the same direction from cage edge out. Time plots were graphed for each transect to show any important features of the series; in this case, we were looking for any change in the immediate vicinity of the fish cages.

Spectral analysis is traditionally used to identify variation (spectral density) at different frequencies (Fourier frequencies). Spectral analysis has two important model assumptions that must be met before the data can be analysed: the first is stationarity and the second is that the data comply with what is commonly know as the frozen turbulence hypothesis. Data is stationary when its mean and variance are constant over the length of the series (Chatfield 1989). If a series is not stationary then the raw data is transformed by either first differencing or detrending before spectral analysis is performed. Secondly, analysis of transect data is said to be complicated by the fact that water and boat are moving in relation to each other (Strutton *et al.* 1997). Taylor's hypothesis, otherwise known as the frozen turbulence approximation, states that if the speed of a probe (U) traversing through a turbulence field is large compared to the velocity field (u) then the media can be regarded as stationary. In this case, U = 1.5 m/s and u ~ 0.4 m/s which satisfies the condition $u << U$ (Strutton *et al.* 1997). This means that the recording of data was not complicated by the movement of the boat in relation to the turbulence field encountered in the water. Each transect was analysed separately which gave 120 power spectral density graphs and the slope was determined for each using linear regression.

Patch definition

Patch definition is a formula developed to measure the intensity, width and amount of phytoplankton patches along the pooled fluorescence transect data. A patch is indicated if fluorescence (RU) is above or below $\pm$ 1SD of the mean of all the transects for each site, on each sampling day,

at a distance (m) along a fluorescence transect. A phytoplankton patch s defined as: $P \geq F(t)-(X)\pm$ 1SD where P is the phytoplankton-patch, F(*t*) is the fluorescence data point at time *t* and mean is the mean of the pooled transects for one site on each sampling day.

Results

Environmental conditions

Temperature at the TC1 and C1 sites for day 2 ranged from 18.2–18.8^{o}C and at the TC2 and C2 sites ranged from 19.1–19.5^{o}C. For day 3 at the TC1 and C1 sites temperature ranged from 18.7–19.3^{o}C and at the TC2 and C2 ranged from 19.8–20.2^{o}C. For day 4 all sites ranged from 18.1–18.5^{o}C. For day 5 at the TC1 and C1 sites temperature ranged from 18.1–18.9^{o}C and at the TC2 and C2 sites ranged from 18.9–19.2^{o}C. As samples were taken in the mornings to very early afternoon there was a shift in surface water temperatures between 1.0 and 1.5^{o}C between sites. However, at each site during sampling the shift in temperature had a minimum 0.4 ^{o}C and maximum 0.8 ^{o}C.

Winds were not strong and ranged between 5 and 10 knots. Seas were reasonably calm ranging from half to 1 m. Days 1 and 4 were strongly overcast 6–7/8 of cloud cover.

Total Chlorophyll *a*

Total chlorophyll *a* (Chl *a*) was obtained by averaging all of the chlorophyll measurements for each site, at 0, 50, and 500 m from all three transects and on all 5 sampling days. Chl *a* ranged from 1.10 to 5.19 μg/L (Table 1) and the mean was higher at C1 (2.77 μg/L) and TC1 (2.75 μg/L) than at TC2 (2.59 μg/L) and C2 (2.48 μg/L). Pair-wise comparisons showed no significant differences in the site/transect treatment (p>0.05) or site/distance treatment (p>0.05). However, there was a significant

difference between site and size (p<0.01). This is explained further in the size fractionation results below. The mean variation of all samples for each site along transects was low.

Table 1: Mean (± 1SE) of total Chl *a* (pooled 0, 50 and 500 m) for all tuna cage sites over 5 sampling days.

Site	**Mean Chl a (± 1SE) (range in parentheses)**	**Sample size (n)**
C1	2.76±0.14 (1.10 to 4.46)	45
C2	2.48±0.94 (1.06 to 4.45)	45
TC1	2.75±0.09 (1.16 to 5.19)	45
TC2	2.59+0.07 (1.09 to 3.48)	45

Table 2 shows the results for tests of between-subjects effects (ANOVA) using total Chl *a* as the dependent variable. When Chl *a* between sites were compared, treatments were significantly different from each other (p=0.05). Chl *a* for TC1 was significantly different to C1, TC2 and C2 (p=0.01) but there was no significant difference between TC2 and C2 (p=0.05). Graphical presentation of Chl *a* samples taken at tuna cages and controls is seen in Figures 4–8. Overall Chl *a* decreased by an order of magnitude from day 1 to day 3 at TC1 and similarly for C1 and then increased to pre-neap tide levels on day 5. This effect was not as distinct at TC2/C2 although the trend was similar.

Table 2: Multiple Comparison of total Chl *a* (pooled 0, 50 and 500 m) for all tuna cage sites over 5 sampling days (n=45).

(i) Site	(j)Site	Mean Difference (I-J)	Sig. (p<0.05)
C1	C2	0.44	0.001
	TC1	-0.51	0.001
	TC2	0.37	0.001
C2	C1	-0.45	0.001
	TC1	-0.96	0.001
	TC2	-7.4932E-02	0.99
TC1	C1	0.51	0.001
	C2	0.95	0.001
	TC2	0.88	0.001
TC2	C1	-0.37	0.001
	C2	7.493E-02	0.99
	TC1	-0.88	0.001

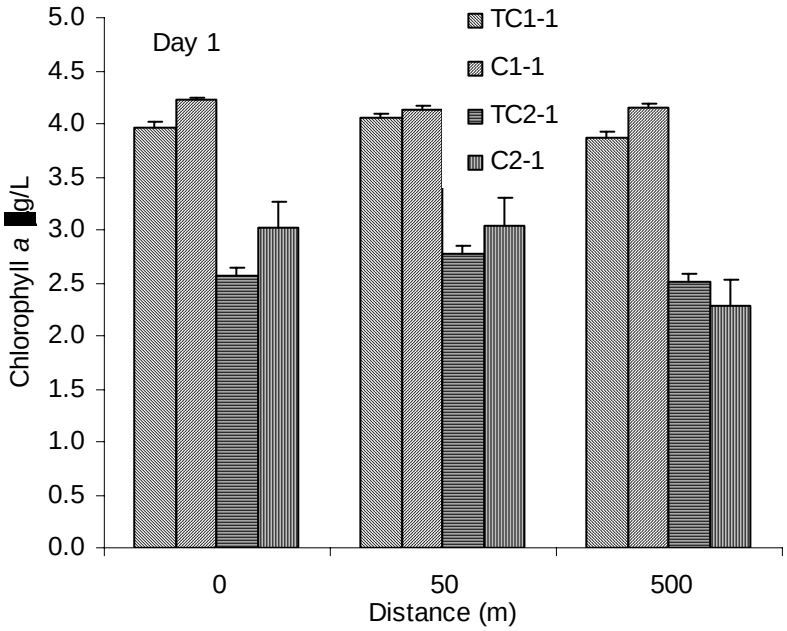


Figure 4: Mean Chl *a* samples taken at tuna cages (TC) and controls (C) on day 1 at 0, 50, and 100 m (± 1SE).

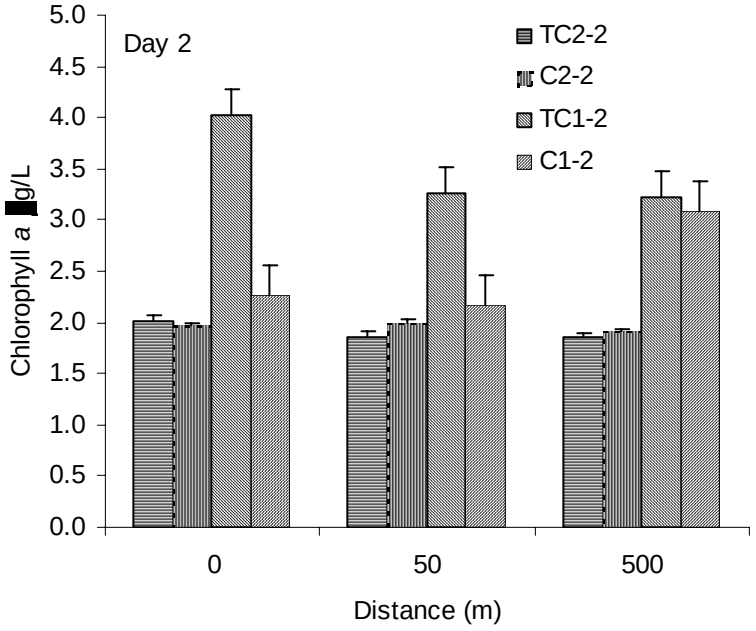


Figure 5: Mean Chl *a* samples taken at tuna cages (TC) and controls (C) on day 2 at 0, 50, and 100 m (± 1SE).

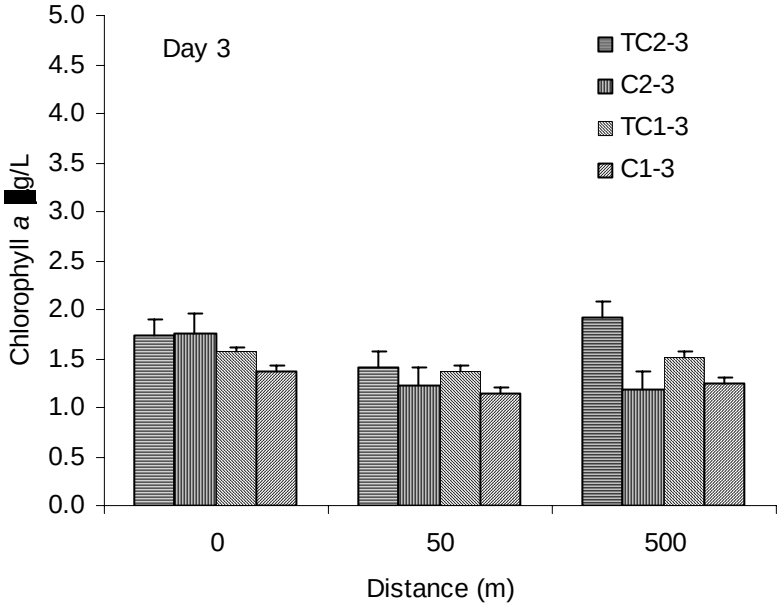


Figure 6: Mean Chl *a* samples taken at tuna cages (TC) and controls (C) on day 3 at 0, 50, and 100 m (± 1SE).

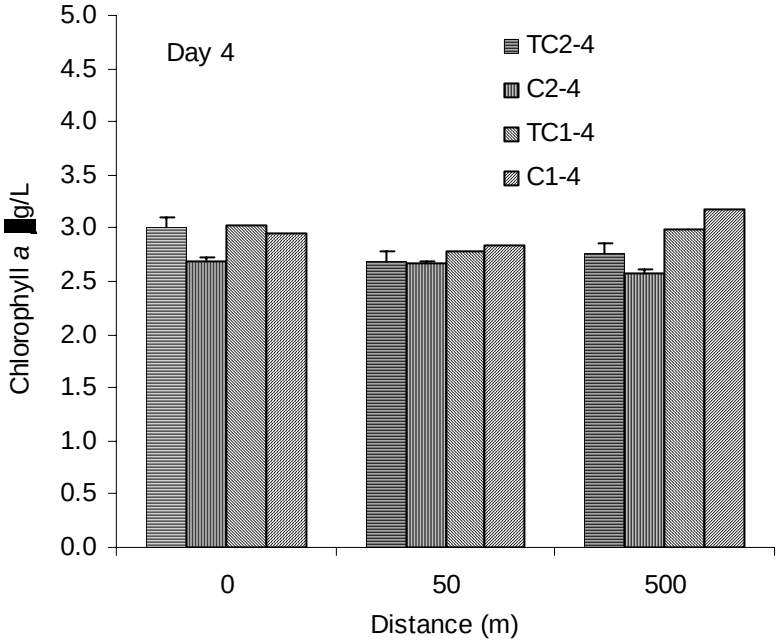


Figure 7: Mean Chl *a* samples taken at tuna cages (TC) and controls (C) on day 4 at 0, 50, and 100 m (± 1SE).

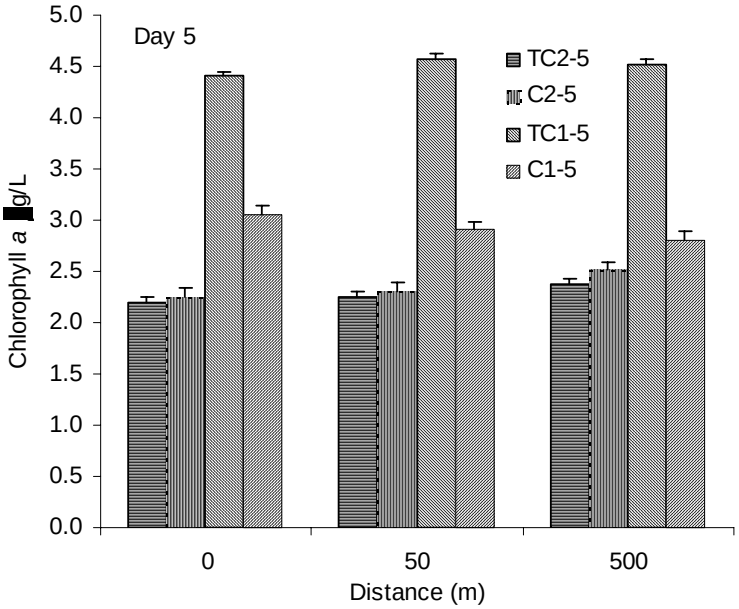


Figure 8: Mean Chl *a* samples taken at tuna cages (TC) and controls (C) on day 5 at 0, 50, and 100 m (± 1SE).

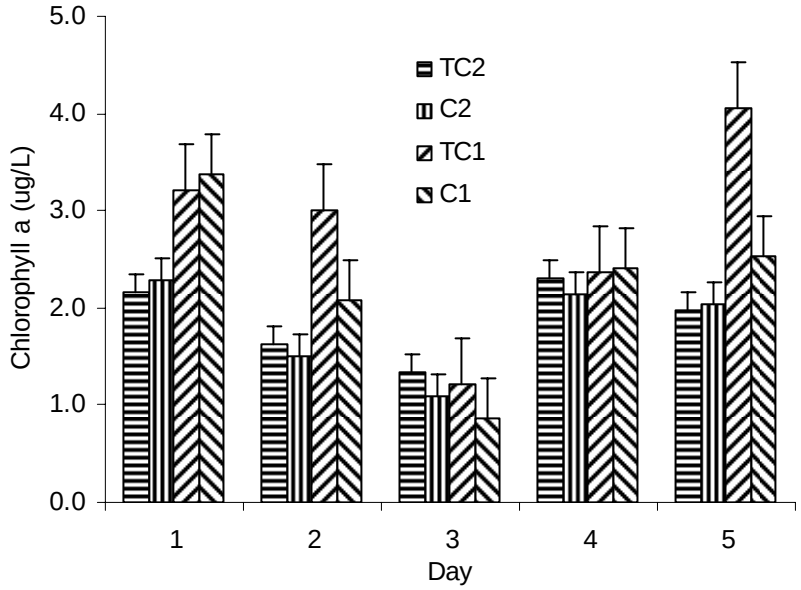


Figure 9: Mean Chl *a* 5μm fraction for each sampling day at tuna cages (TC) and controls (C) (± 1SE).

Chlorophyll a – 5 μm fraction

The 5 μm fraction for each sampling day (Figure 9) was larger at TC1 and C1 on days 1, 2 and 5 than at TC2 and C2. Size fractionated Chl *a* were analysed separately by computing multiple comparisons. The larger fraction of Chl *a* was found in the 5 μm fraction (ranged from 62% at C1 to 74.3% at TC2, of total chlorophyll *a*). A significant difference was recorded for the combination TC1 and C1 indicating a strong affect of Chl *a* at the larger fraction of 5.0 μm between TC1 and its control site C1 (p<0.01). A significant difference was recorded between the site areas TC1/C1 and TC2/C2 (p<0.01). However, no significant difference was found between TC2 and C2. Pair-wise analysis of the total 5 μm fraction, for all transects on all sampling days, within the tuna cage and control sites, did not reveal

any significant difference. However, significant differences appeared when the site, treatment and distances were compared (p<0.05).

Chlorophyll a – 0.45 μm fraction

The 0.45 μm fraction for each sampling day (Figure 10) was larger at TC1 and C1 on days 1 and 5 than at TC2 and C2. The 0.45 μm fraction was the smaller of the fractions (ranged from 25.7% at TC2 to 38% at C1, of total Chl *a*). A significant difference was recorded between the site areas TC1/C1 and TC2/C2 (p<0.01). However, no significant differences were found between TC1 and TC2 (p=0.06) and C1 and C2 (p=0.07) or between tuna cages and controls (TC1/C1, p=0.46; TC2/C2, p=0.18).

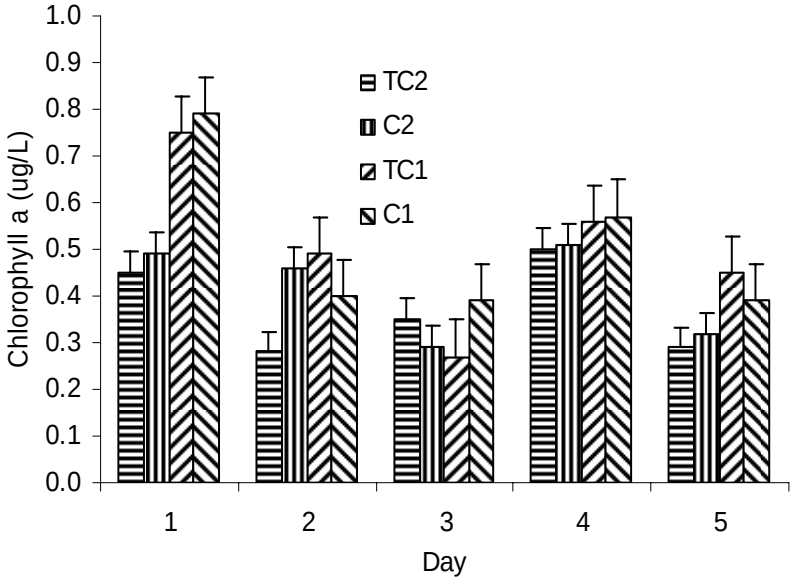


Figure 10: Mean Chl *a* 0.45 μm fraction for each sampling day at tuna cages (TC) and controls (C) (± 1SE).

Tests of between-subjects effects of ranked Chl *a* in the 0.45 μm fraction revealed a strong response with size along the transects (p<0.05).

Further dissection revealed that Chl *a* in the 0.45 μm fraction was significantly lower in the 50 m samples than in the 0 and 500 m samples ($p<0.05$). However, this appeared as an overall random response amongst sites, directions and sampling days.

Fluorescence signal along directional transects

Figure 11 is an example of a time plot for temperature and fluorescence (TC2-3-W1 and W2; W=westerly transect). Temperature showed no variation over the short transects compared to florescence, this was evident in all 120 transects recorded. There were no obvious changes in fluorescence associated with temperature. Some transects show higher fluorescence at the tuna cage edge (0–100 m) and this is evident on days 1, 2, 3 and 5 at TC1 and 1, 2 and 3 at TC2. Variations in fluorescence along transects are not large (see Table 4 for ranges). Control site fluorescent transects were mostly flat, whilst the tuna cage transects appeared to have higher wavelength than the controls. This was analysed further by transforming the raw transect data for day 1 using a log normal transformation, and creating separate time plots.

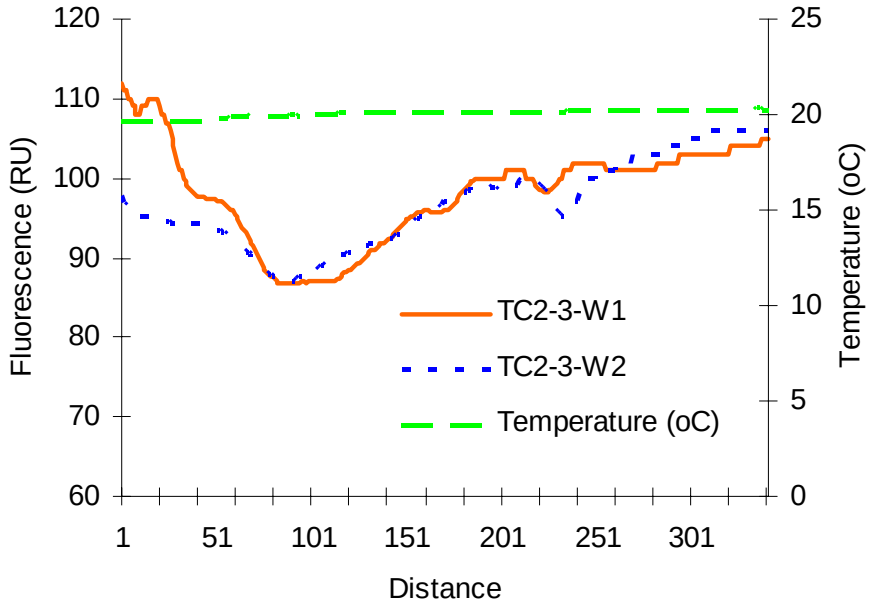


Figure 11: Example of a time plot for *in vivo* fluorescence (RU=relative units) transects and temperature (^{o}C) for TC2 on day 3, in the westerly direction (TC=tuna cage; W1 and W2=westerly replicate transects).

Wavelengths for the western transects on day 1 at the tuna cages ranged between 7 and 9 whereas for the controls ranged between 2 and 5. Transects at the TC1 site appear to have an overall higher fluorescence than the other sites. Replicate transects (1 and 2) mostly reflect each other; there are some exceptions for example, C1-1-W where W1 is substantially different from W2.

Statistical analysis of fluorescence transect data

Table 3 shows an example of the range of fluorescence for all sites on day 1, for the north one (N1) transect. The fluorescence data for all transects was restricted to 400 points on the distance scale, then daily transects for each site were treated as replicates. There was a distinct

difference in fluorescence signal between the site areas of TC1 and TC2. Univariate analysis of variance of pooled transects for each site of *in vivo* fluorescence around the tuna cages showed significant effects on the days 1, 2 and 5. Paired comparisons were used to determine pairs of means that differed significantly. TC1/C1 differed marginally on day 1 from all other sites but was not significant (p=0.08) and TC1 differed significantly from all other sites on day 2 and 5 (p<0.05).

Table 3: Example of range, mean (± 1SE) and sample size of fluorescence (RU=relative units) for all sites on day 1, north one (N1) transect only (TC=tuna cage; C=control).

Site/Day/Direction/Transect	Mean fluorescence (RU) (± 1SE) (range in parentheses)	Sample size (n)
TC1-1-N1	228.9±0.7 (217-270)	296
TC2-1-N1	176.2±0.32 (160-197)	296
C1-1-N1	210.2±0.6 (202-222)	296
C2-1-1-N1	143.3±0.85 (128-168)	296
TC1-2-N1	206.2±0.25 (2.1-222)	339
TC2-2-N1	110.6±0.29 (107-134)	340
C1-2-N1	125.3±0.06 (123-128)	340
C2-2-1	118.35±0.3 (110-128)	340
TC1-3-N1	103.4±0.44 (91.7-122)	340
TC2-3-N1	89.2±0.27 (84.7-103)	340
C1-3-N1	84.8+0.04 (83.5-87.7)	340
C2-3-N1	89.8±0.06 (88.3-94.1)	340
TC1-4-N1	181.4±0.16 (176-188)	340
TC2-4-N1	170.7±0.44 (161-206)	400
C1-4-N1	203.5±0.27 (197-226)	344
C2-4-N1	161.0+0.45 (150-188)	400
TC1-5-N1	188.1+0.4 (182-217)	400
TC2-5-N1	158.1+0.37 (142-171)	400
C1-5-N1	149.2+0.27 (143-165)	424
C2-5-N1	147.7+0.2 (140-153)	387

Response of in *vivo* fluorescence within 50 m of the tuna cages

In this analysis the first and last 50 m of the fluorescence transect data was used to show if effects were similar at both ends of the transects. Within site transects were pooled and compared for each day. Table 4 shows that within the first 50 m there were 3 days that were significantly different from each other and these were on the days prior to and after the neap tide events (p<0.01). Significant differences were found in the last 50 m of data on days 2 and 5 (p<0.05).

Table 4: Results of univariate analysis of variance comparing between effects of the first 50 m of the fluorescence transects and between effects of last 50 m at tuna cages (TC) and controls (C).

Day	First 50 m (Sig p<0.05)	(I)Site	(J)Site	Sig. (p)	Last 50 m (Sig) (p)	(I)Site	(J)Site	Sig (p)
1	0.01	TC1	C1	0.305	0.102	TC1	C1	ns
			TC2	0.033			TC2	ns
			C2	0.014			C2	ns
2	0.00	TC1	C1	0.01	0.002	TC1	C1	0.06
			TC2	0.01			TC2	0.005
			C2	0.01			C2	0.004
3	0.08	TC1	C1	0.08	0.283	TC1	C1	ns
			TC2	ns			TC2	ns
			C2	ns			C2	ns
4	0.456	TC1	C1	ns	0.407	TC1	C1	ns
			TC2	ns			TC2	ns
			C2	ns			C2	ns
*5	0.00	TC1	C1	0.01	0.018	TC1	C1	0.02
			TC2	0.01			TC2	0.1
			C2	0.01			C2	0.05

Furthermore, when paired comparisons of means were made, TC1 and C1 were, as an area, significantly different from TC2 and C2 but not

significantly different from each other. When multiple comparisons where made, based on observed means, TC1 had a significant impact on the observed differences between the sites on days 1, 2 and 5 in the first 50 m surrounding the cage and on days 2 and 5 in the last 50 m.

Phytoplankton abundance

Nanophytoplankton, flagellates, diatoms and small coastal gymnoids dominated the phytoplankton counts during this study. *Leptocylindricus danicus*, *Pseudonitzschia* spp., *Chaetoceros* spp. *Hemiaulus* sp., *Eucampia zoodiacus* and *Cerataulina bergonii* dominated the diatoms, ranging from approximately $3.9x10^5$ cells/L for *L danicus* to $0.7x10^4$ cells/L for *C. bergonii*. Nanophytoplankton were by far the most abundant organism, ranging from $>2.5x10^6$ cells/L to $4.6x10^6$ cells/L. Potentially harmful algae were rare. *Karenia mikimotoi*, for example, was present in all samples at $<3.4x10^2$ cells/L. Species composition did not change spatially or temporally on any of the sampling days but the abundance of the dominant organisms matched the movement of the chlorophyll *a* levels.

Spectral analysis

Spectral analysis was performed on the transect data to determine if we could use this method to detect whether effects measured around the tuna farms were directly attributable to biological or physical processes. The data was transformed using the first differencing function to satisfy the assumption of stationarity. Spectral densities were plotted vs frequency

and linear regression performed. The logarithmic power spectra for the fluorescence and temperature time series together with their best fitting lines are given in Figure 12. Linearity is evident at only the smaller scales for fluorescence but stronger for temperature. This is most likely a product of the short distance of the transects.

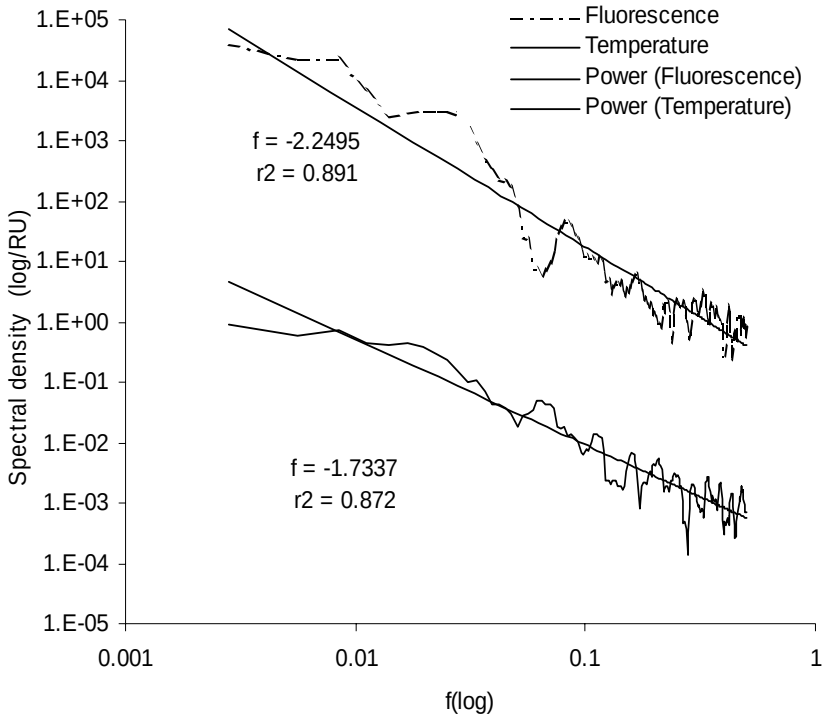


Figure 12: Example of power spectra (f is frequency) of the fluorescence time series (log/ RU=relative units) and temperature time series for north 1 transect at C2 (control) on day 5, shown with their best-fitting lines in a log-log plot. Coefficient (r^2) ranged from 0.75 to 0.97.

The slopes of the power spectra for fluorescence ranged from 0.00 to 0.68 on day 1 to 0.018 to 2.24 for day 5. Small patches were evident in the 0–100 m range. The slopes of the temperature spectra ranged between 1.41 to 1.73. Power spectra detect periodicity, so if it is a single or a few

patches, or an aggregate of patches that are randomly distributed and sized then the power spectra will not detect them and is therefore not the best technique to indicate patches.

Patch definition

Results show phytoplankton patches were higher at the TC1 edge (0–100 m) than at C1 (Figure 13) and C2 on days 1, 2, 3 and 5 and at TC2 on days 1, 2, and 3.

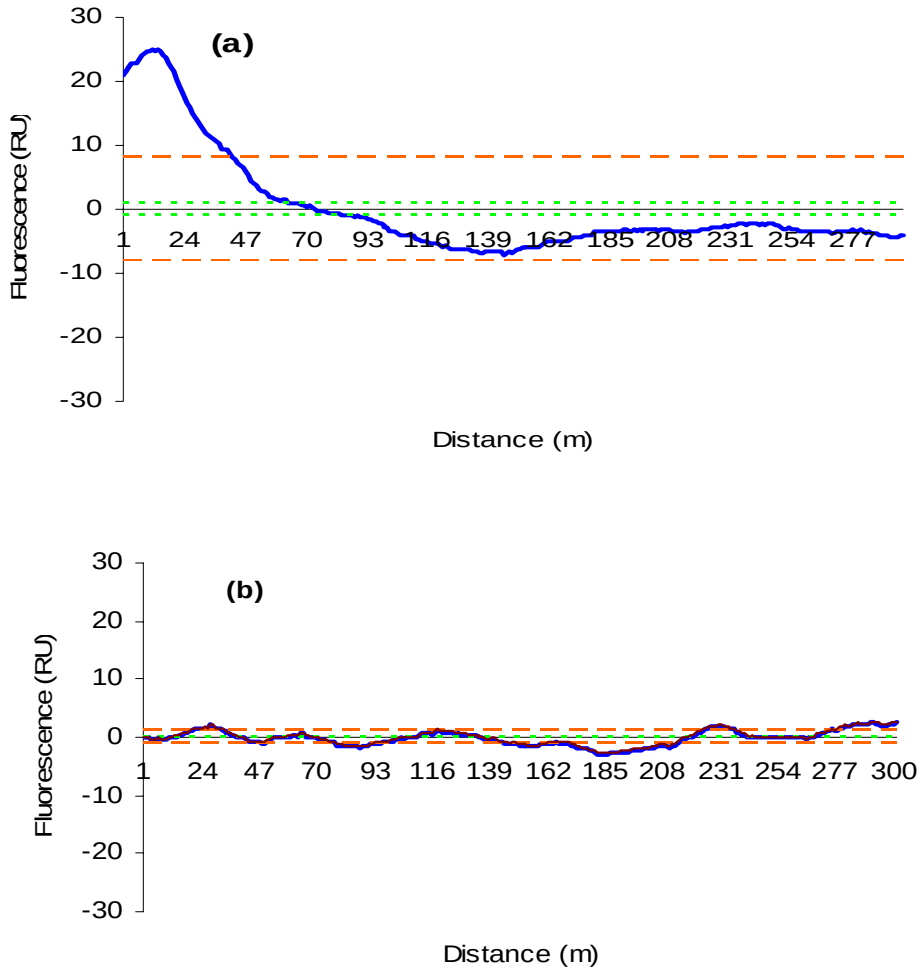


Figure 13: Example of patch definition at TC1 (a) and C1 (b) on day 1, pooled and detrended fluorescent (RU=relative units) transect data (blue line=TC1; broken orange line=± 1SD; green dotted line=95% CL).

Using this method few patches were evident along any of the transects. Patches were evident at TC2 as well as TC1, which were not apparent in either the chl *a* data or statistical analysis of the fluorescent transect data.

Discussion

Fish farmers are generally interested in water quality as it relates to the health of their stock and proclivity of toxic organisms but not to the regional ecology. Institutions that are employed to take water quality parameters in fish farming regions often do not have the resources to extract ecologically relevant samples (high temporal and spatial resolutions) to determine the underlying effects of fish farm activities. This is the first time that ecologically relevant temporal and spatial scales of sampling phytoplankton around fish farms have been attempted in South Australia. We are aware that this study only provides a small window in time, however, it has shown that using continuous flow *in vivo* fluorescence we could detect persistent phytoplankton patches of a particular size, temporally and spatially, around tuna cages in an area equivalent to >1000 m^2 over a 15 day period. Unlike other studies around fish farm sites this study also establishes that low sampling resolution horizontally and statistical pooling of data is unlikely to deliver ecologically sensitive results for monitoring programs.

Observation of fluorescence time-plots revealed areas of higher fluorescence on 4 out of the 5 sampling days in the first 0–100 m of TC1, and on 3 out of the 5 sampling days at TC2 (Figure 11). Control fluorescent time plots were mostly flat with a few transects showing high fluorescence randomly along the transects. Comparatively, total chlorophyll *a* was significantly different at TC1 when compared to all other sites (Table 2) on the days prior to and after the neap tide events and

there was a significant difference between the 0, 50 and 500 m samples along the transects on 2 out of the 5 sampling days. Further, statistical analysis of the pooled fluorescence transect data revealed significant effects on days 1, 2 and 5 for the area of TC1, effectively dampening the observations of patches in the time plots, particularly at the TC2 site. Indeed, pooling the data removed the temporal variability over days from the data and lead to the erroneous conclusion that there were no patches at the tuna cage edge at the TC2 site or during the neap tide events.

The dominance of the TC1 site in the statistical analysis was due to the overall high fluorescence at this site, which was further elucidated when comparisons were made of the first 50 m of the transects with the last 50 m using univariate analysis of variance. Table 4 shows that there were significant differences in the first 50 m between TC1/C1 and TC2/C2 and the last 50 m of the transects on 3 and 2 days respectively out of the 5 sampling days. The site TC1 had a significant impact on the observed difference between the sites within the first 50 m on days 1, 2 and 5 and on the last 50 m on days 2 and 5.

Spectral analysis of the fluorescent transect data showed that not only small patches were dominant but that the distribution of the largest patches were within 100 m of the tuna cages (Figure 12). Results from the patch definition exercise showed that this was the case and that there were patches at both of the tuna cages within the first 0–100 m of the cage edge (Figure 13), the intensity of which was larger at the TC1 site. Some

anomalies also appeared during this study, such as that the fluorescence and chlorophyll signals were dampened 2-fold at all sites during the neap tide events. In addition, results from the total chlorophyll *a* and size fractionation analysis showed that control sites were generally similar to the tuna cages sites with the exception on some days at TC1. How do we account for these differences?

There was no correlation between chlorophyll/fluorescence variability around the tuna cages and the physical processes of temperature and salinity. This was expected over such short transects as uncoupling of physical processes with biological processes is not uncommon at length scales of <5 km (Platt and Denman 1975; Abbott *et al*. 1982; Abraham 1998). Therefore, salinity and temperature were not responsible, spatially or temporally, for the observed changes in chlorophyll/fluorescence during this study.

Particular size characteristics of phytoplankton often drive responses in the marine environment (Cottingham 1999). In oligotrophic waters and open ocean waters microphytolankton are often responsible for approximately 30% of the chlorophyll contribution with pico- and nanophytoplankton making up the other 70% (Jiao and Wang 1994). Shifts from smaller cell sizes to larger cells have been observed in areas of nutrient enrichment (Yew-Hoong Gin *et al.*2000; Jacquet *et al.* 2006). Shifts from small to large cells with increasing nutrient enrichment may be explained by the capacity of larger cells to multiply faster than smaller cells

when not nutrient limited (Agawain *et al.* 2000; Fernandez *et al.* 2003). It is also hypothesized that a shift from small to large cells is enhanced by lower predation rates on larger cells in nutrient enriched conditions (Jacquet *et al.* 2006).

The standing stock of cells was dominated by the pico- and nanoplankton size class and the larger size class of phytoplankton, >5.0 μm, was driving the chlorophyll response, particularly at the TC1 site, during this study. Similarly Yew-Hoong *et al.* (2000) have shown for coastal waters of Singapore measurements of size fractionated chlorophyll show that the chlorophyll contribution of the picoplankton and nanoplankton size classes (<5.0 μm) was generally smaller than that of the microplankton. These findings are consistent with the higher nutrient levels in coastal waters (Malone 1980; Yamamoto and Taneguchi 1993; Jiao and Wang 1994) compared with open ocean waters (Legendre *et al.* 1993).

The diatoms *Leptocylindricus danicus, Pseudonitzschia* spp., and *Chaetoceros* spp., were dominant in this fraction. Evidence of changes in size structure in response to nutrient inputs have been shown in freshwater lakes (Cottingham 1999) and coastal marine and estuarine systems (Iriarte and Purdie 1994; Han and Furuya 2000). In a study on the southwestern lagoon in New Caledonia it was found that picophytoplankton-dominated assemblages were replaced by microphtoplankton-dominated assemblages with increasing dissolved

inorganic nitrogen concentrations from anthropogenic sources. Diatoms were the dominant microphytoplankton species and it was concluded that nutrient enrichment of the bay favors large diatoms at the expense of coccolithophorids and dinoflagellates (Jacquet *et al.* 2006). This is in contrast to the hypothesis put forward by Smayda (1990) where it was suggested that under nutrient ratio changes dinoflagellates would be favored. This study was very short-term so we are unable to say that there was a shift from small to large cells within the phytoplankton population in Boston Bay; however, it appears that larger diatoms were the main contributors to the higher chlorophyll response at the TC1 site. This may be a strong indication of a response to nutrient inputs. Further research using a finer resolution of class sizes and nutrient measurements may determine if this is the case.

At the TC1 site nutrient inputs come from fish farm waste and sediment under the cages. The TC1 site, during this study, had three 50 m diameter cages joined together and had been in place for four years with a lay-off period each year (during which no fish are held in the cages) of approximately 2-3 months. Cages were in only 16 m of water. Not only had large amounts of sediment accumulated under the cages over the four-year period for which the scouring area under any one cage may extend to approximately 100 m (Cheshire *et al*. 1996) but also large amounts of soluble N are poured into a fish farm area daily. Approximately 5,000 t of tuna is farmed in Boston Bay and Spencer Gulf from late November to around September of the following year. The tuna are fed

approximately 260 t of pilchards per day, which is 9% of body weight per day. Using the feed conversion ratio, 15 t of tuna would produce approximately 245 t of waste per day, of which 60–70% is urinary ammonia and urea (Smart 1996). Future work may look for the long-term response of size structure to nutrient inputs in this system.

Chlorophyll and fluorescence signals were suppressed during the neap tide events which resulted in a homogenous effect at all sites. Boston Bay waters are well mixed and the combination of surface winds and tidal movement will affect currents (turbulence) dramatically. Studies have shown that chlorophyll often increases during an incoming (or flood) tide and decreases during the outgoing tide (Seuront *et al.* 2002). Patches are maintained by a combination of mechanisms: these are many and include turbulence, physico-chemical parameters such as temperature, salinity and nutrient distributions, behavioral characteristics such as swimming and swarming of organisms, and reproduction and predator/prey interactions. However it is considered that the process of diffusion due to turbulence and random movements of organisms tend to counteract the formation of patches and gives rise to more uniform distributions (Steele and Henderson 1994).

A period of turbulent weather will cause the surface water to be mixed into the bottom layers, particularly if this is also the wind direction. Patches of phytoplankton move on rapidly in turbulent environments. It has been found that the presence of density gradients (stratification) may restrict the

size of eddies that turn over vertically so that mixing is greater in the horizontal than in the vertical direction (Denman and Gargett 1995). Depending on wind speed, current shear and stratification, time scales for cycling phytoplankton by turbulent eddies and mixing in the sea have been found to vary from about 0.5 to 100 h for vertical displacements of 10 m. The mixing response of the surface layer to the onset of strong wind is rapid, a few hours, and quickly reduces cycling times by turbulent eddies to less than 1 h (Denman and Gargett, 1995). Boston Bay is a well-mixed environment with no stratification detected in this or prior studies, so there are no density gradients, and mixing may take place in the vertical depending on wind speed and currents. Sampling was carried out during calm weather in the mornings but in the afternoons, on-shore, south easterly winds had sprung up (15 to 20 knots) on days 3 and 4, which effectively mixed the surface layer through turbulent action despite the lack of water movement caused by the neap tides.

Additionally, it is possible that the tuna cages shed fouling phytoplankton from their surfaces. However, the patches were not always against the cage. This may be because the phytoplankton associated with the net surfaces may be in the deeper portion of the cage to minimize photoinhibition and to take advantage of dissolved nutrients emanating from sinking food and feces. As water passed by phytoplankton would be removed, and because the water is well mixed, they would be homogenized over the water column and create a patch at a distance of

tens to hundreds of meters from the cage. Future work might look for this halo patch around cages.

Cage dynamics may also affect phytoplankton in the surrounding waters in different ways. If the cage is visualised as a grid, fluid dynamics dictates that a certain amount of turbulence is caused by the shape of the grid as water moves through it (Vogel 1994). There was an indication of this in the larger wavelengths common on the western transects at the tuna cage sites. The diffusion of water is also slowed through the cages caused by heavy benthic growth on the ropes, and their small pore size (Beveridge 1996). Combined with bottom currents in Boston Bay, this may serve to resuspend nutrients from sediments beneath the cages and generally to act as a trap for phytoplankton in the area of the cage long enough for reproduction to take place in the farm area.

Conclusion

This study has provided a small window in time of dynamics around tuna fish farms in Boston Bay. The results from the chlorophyll *a* sampling show little spatial variability within a site in the 1000 m^2 that the sampling area covers but far greater temporal variability (days). In contrast, fluorescence `mapping' expands the window of variability both spatially (within a site) and temporally (along transects and between days). This has given a spatial definition, which is unavailable from a single point sample and thereby, leaves room for much greater interpretation. Small

patches are evident from the fluorescence mapping where this is impossible to detect from the single point samples. Therefore, the fluorescence `mapping' and patch definition shows that the trend is widespread (spatially) and quite persistent (temporally) around the fish farm area.

Size fractionated chlorophyll samples provided further insight into phytoplankton dynamics in this study where diatoms were favored over dinoflagellates and were responsible for the larger fraction of chlorophyll found at the TC1 site. We suggest that seasonal fluctuations, high nutrient input from the farm activities, and turbulence may be responsible for the different chlorophyll/fluorescent structures found at TC1. Future research may look at the long-term, regional impact on phytoplankton size structure, biomass and communities from fish farm activities.

Chapter 5

This is the first study in South Australia to identify phytoplankton species distribution and abundance, including two potentially toxic dinoflagellates, to define the short-term biomass fluctuations of chlorophyll and *in vivo* fluorescence and to describe patch distribution relative to tuna fish farms in Boston Bay and the near-shore waters of lower Spencer Gulf. An ecological interpretation of phytoplankton distribution and abundance is determined and shows that community composition was different in lower Spencer Gulf compared to Boston Bay and upper Spencer Gulf sites. Pico- and nanoplankton were often the most abundant organisms. Diatoms and gymnoids were most common. Season and currents predominantly influenced distribution of phytoplankton in Boston Bay and Spencer Gulf. Individual species may be influenced by inputs from the fish farms. As a good part of this journey involved counting phytoplankton using the Utermöhl technique, a short paper was published in the Journal of Plankton Research on reducing the settling time of this method. (This is presented in Appendix 2.)

Summary of chapters

Chapter 2

The physical water quality parameters followed seasonal patterns and were similar at tuna cages and controls (Figure 2). Average ammonia concentrations were generally higher in 1998 than in previous years but ammonia was not significantly different between tuna cages and controls. DO concentrations were generally opposite that of ammonia during the autumn months. Phytoplankton blooms in Boston Bay were most obvious

in the autumn months; however, blooms of different species occurred seasonally. Overall there appeared no statistically significant effect between tuna cages and controls for phytoplankton biomass (chlorophyll *a*) in Boston Bay or surrounds. However, there was a significant effect of chlorophyll *a* between the area of lower Spencer Gulf, where there were no tuna farms, and all other areas. There was also a significant effect of chlorophyll *a* between years and between seasons. This effect was observed most strongly on the eastern side of Boston Island. Similarly, the Principal Components (PC) analysis showed that there was no statistically significant effect from phytoplankton counts between fish farm sites and controls. The main effect found was from season and area. Of the 131 taxa identified fourteen were dominant and 4 PC were extracted (Table 1 and 2). Factor loadings were principally related to taxa suites that varied in their time of blooming (Figures 6-9).

Functional classification of dinoflagellates (Smayda and Reynolds 2001), aided by principal components analysis, added another dimension of classification into the habitat distribution of phytoplankton found in Boston Bay and near-shore lower Spencer Gulf. Potentially toxic phytoplankton identified during this study included: *Karenia brevis, K. mikimotoi, Dinophysis acuminata, D. acuta, Heterosigma* spp.*, Ceratiurm fusus, Pseuidonitzschia* spp., *Chattonella marina* and *Alexandrium minutum*. Picoplankton was often the most abundant organism with *Cylindrotheca closterium* and *Guinardia* spp., the most abundant diatoms, and the smaller gymnoids the most abundant dinoflagellates during this study (Figures 10–14). Diatoms dominated phytoplankton community

composition in the Spencer Gulf samples: dinoflagellates were predominant in samples east of Boston Bay Island and almost equal proportions of diatoms and dinoflagellates were found in Boston Bay samples (Table 3).

Chapter 3

K. brevis and *K. mikimotoi* look-alikes from Boston Bay and Spencer Gulf are described. *K. brevis* production peaked in late summer and autumn of 1997/98, with the highest recorded numbers found at sites near the entrance to Proper Bay. *K. brevis* was the dominant organism during summer and autumn. *K. brevis* blooms did not reach what is defined as `fish killing proportions' (1-2.5x10^5 cells/L) (Tester and Steidinger 1997) in Boston Bay and there were no recorded deaths of marine organisms due to toxicity. Samples from the Lower Spencer Gulf sites contained no *K. brevis* or no cells in detectable numbers except during March 1998 where cells numbers were 7x10^2cells/L. They began to disappear in September and November 1998 and did not appear in such numbers during 1999 or 2000 (SA Water unpublished data). No significant differences for *K. brevis* cells were found between tuna cages and controls.

K. mikimotoi bloomed generally from May or June to November or December in 1997 and 1998. However, numbers at the SG1 TC in upper Spencer Gulf sites, area 4, increased in April 1998 from <1x10^2cells/L to 1.5x10^4 cells/L and decreased to undetectable levels in November. *K. mikimotoi* numbers were highest (4.5x10^4cells/L) at BB12 in area 2 (western side of Boston Bay) in Sept/Nov 1997 and did not reach these

numbers in 1998. *K. mikimotoi* was the dominant organism during 1997 but not 1998. Samples from the lower Spencer Gulf sites contained few *K. mikimotoi* or cells in undetectable numbers except during November 1997 cells were detected at $2.5x10^2$cells/L, in March 1998 $1.4x10^3$cells/L and June 1998 at $2x10^2$cells/L. Significant differences for *K. mikimotoi* cells were found between tuna cages and controls and these were distributed mainly at cages around Boston Island.

Chapter 4

Observation of fluorescence time-plots revealed areas of higher fluorescence on 4 out of the 5 sampling days, in the first 0–100 m of TC1 and on 3 out of the 5 sampling days at TC2 (Figure 11). Control fluorescent time plots were mostly flat with a few transects showing high fluorescence regions distributed randomly along the transects. Comparatively, total chlorophyll *a* was significantly different at TC1 when compared to all other sites (Table 2) on the days prior to and after the neap tide events and there was a significant difference between the 0, 50 and 500 m samples along the transects on 2 out of the 5 sampling days. Further, statistical analysis of the pooled fluorescence transect data revealed significant effects on days 1, 2 and 5 for the area of TC1 effectively dampening the observations of patches in the time plots particularly at the TC2 site.

Spectral analysis and patch definition of the fluorescence transect data showed that small patches were dominant and that the distribution of the largest patches was within 100 m of the tuna cages (Figure 12 and 13).

The intensity of fluorescence patches was largest at the TC1 site. There was no correlation between chlorophyll/fluorescence variability around the tuna cages and temperature and salinity. Size fractionated chlorophyll results showed significant differences between the site areas of TC1/C1 and TC2/C2 for both the 5.0 and 0.45 μm fraction (Figures 9 and 10). There was also a significant difference in the 5.0 μm fraction between TC1 and C1. No significant differences for either of the fractions were found between the sites TC2 and C2. Overall results were similar to that of total chlorophyll and fluorescence. Chlorophyll and fluorescence signals were suppressed during the neap tide events which resulted in a homogenous effect at all sites on days 3 and 4.

Discussion

Of the various effects from fish farming, the most problematic world wide are local and regional eutrophication, phytoplankton community disruption and sediment accumulation. All of these are complexly linked through the vagaries of differential environmental parameters, spatial and temporal patterns and, most significantly, water movement and turbulence. Without a basis for comparison ,small shifts in ecological process such as community structure of phytoplankton or biomass, caused by activities such as fish farming, are easy to miss. To avoid missing those changes it is important to track small details over long periods. It begins with baseline studies of phytoplankton and biomass, particularly in relation to anthropogenic inputs. This study fills that initial requirement for Boston Bay and lower western Spencer Gulf. Several indicators of change were used: environmental parameters, ammonia, chlorophyll (biomass) and size fractionated chlorophyll, phytoplankton abundance and distribution of communities and individual species, and *in vivo* fluorescence.

Environmental parameters

In both studies (chapter 2 and 4) the environmental parameters were not linked spatially or temporally to specific sites or the dynamics of chlorophyll or fluorescence. In chapter 2 we showed that temperature, salinity, pH and dissolved oxygen in Boston Bay and Spencer Gulf displayed seasonal responses with no effect at tuna cages or controls. Further, there was no correlation between chlorophyll/fluorescence variability along directional transects around the tuna cages and the physical processes of temperature and salinity (chapter 4). This was

expected over such short transects as uncoupling of physical processes with biological processes is not uncommon at length scales of <5 km (Platt and Denman 1975; Abbott *et al*. 1982; Abraham 1998).

Chlorophyll

Discrete sampling methods taken only monthly or 6 weekly as in the first study (chapter 2) may serve to obscure localised effects. Chlorophyll *a* levels across all sites in Boston Bay for the autumn months were mostly above the range for oligotrophic waters in a temperate environment. The Spencer Gulf sites were usually below the 1 μg/L specified (ANZECC 2000) and average chlorophyll *a* levels showed seasonal responses with no significant differences between tuna cages and controls (chapter 2). Recently it is thought that studies seeking to determine if blooms are related to fish farm activities should focus on the period within the annual cycle when harmful blooms are likely to develop (Smayda 2006). For Boston Bay waters this would appear to be autumn/winter. If we follow the hypothesis applied to Scottish waters, then during this post-summer period, nutrient levels particularly N, are lowered and limit the growth of phytoplankton. Additional N, therefore, particularly in N sensitive systems is expected to stimulate phytoplankton growth. Fish farms are a source of this nitrogen (Smayda 2006).

Responses during the autumn months showed some differences at individual sites, particularly in the March 1999 samples. Past studies in the area also indicate that levels of chlorophyll around some tuna cage sites were higher than controls (Paxinos *et al.* 1996; Clarke 2000). In chapter 4,

using higher temporal and spatial resolution of sampling, we showed that during autumn of 1999 where sampling was intense (every three days), chlorophyll levels were particularly high at TC1 within the first 100 m of the cage edge in all directions, north, south, and west of the tuna cage.

Particular size characteristics of phytoplankton often drive responses in the marine environment (Cottingham 1999). In oligotrophic waters and open ocean waters microphytoplankton are often responsible for approximately 30% of the chlorophyll contribution with pico- and nanophytoplankton making up the other 70% (Jiao and Wang 1994). Shifts from smaller cell sizes to larger cells have been observed in areas of nutrient enrichment (Gin *et al.* 2000; Jacquet *et al.* 2006). Shifts from small to large cells with increasing nutrient enrichment may be explained by the capacity of larger cells to multiply faster than smaller cells when not nutrient limited (Agawain *et al.* 2000; Fernandez *et al.* 2003). It is also hypothesized a shift from small to large cells is enhanced by lower predation rates on larger cells in nutrient enriched conditions (Jacquet *et al.* 2006). In chapter 4 we demonstrated that the standing stock of cells was dominated by the nanophytoplankton. Clearly the larger size class of phytoplankton, >5.0 μm, was driving the chlorophyll response, particularly at the TC1 site, during this study. Similarly Gin *et al.* (2000) have shown that for coastal waters of Singapore measurements of size fractionated chlorophyll show that the chlorophyll contribution of the picophytoplankton and nanophytoplankton size classes (<5.0 μm) was generally smaller than that of the microphytoplankton. These findings are consistent with the

higher nutrient levels in coastal waters (Malone 1980; Jiao and Wang 1994) compared with open ocean waters (Legendre *et al.* 1993).

The diatoms *Leptocylindricus danicus, Pseudonitzschia* spp., and *Chaetoceros* spp., were dominant in this fraction. Evidence of changes in size structure in response to nutrient inputs have been shown in freshwater lakes (Cottingham 1999) and coastal marine and estuarine systems (Iriarte and Purdie 1994; Han and Furuya 2000). In a study on the southwestern lagoon in New Caledonia it was found that picophytoplankton-dominated assemblages were replaced by microphtoplankton-dominated assemblages with increasing dissolved inorganic nitrogen concentrations from anthropogenic sources. Diatoms were the dominant microphytoplankton species and it was concluded that nutrient enrichment of the bay favors large diatoms at the expense of coccolithophorids and dinoflagellates (Jacquet *et al.* 2006). This is in contrast to the hypothesis put forward by Smayda (1990) where it was suggested that under nutrient ratio changes dinoflagellates would be favored. This study was very short-term so we are unable to say that there was a shift from small to large cells within the phytoplankton population in Boston Bay however it appears that larger diatoms were the main contributors to the higher chlorophyll response at the TC1 site. This may be a strong indication of a response to nutrient inputs. Further research using a finer resolution of class sizes and nutrient measurements may determine if this is the case.

Thus size fractionated chlorophyll measurements taken at a high spatial and temporal resolution result in measurements of biomass that are ecologically relevant, and from this study give an indication of local eutrophication at particular fish farm sites. It is said that a dilemma often exists between sampling scale and the ecologically relevant scale which may result in inaccurate interpretation of phytoplankton ecology (Waters 2003). Largely, this occurs when using discreet sampling techniques particularly at low spatial and temporal resolution. *In vivo* fluorescence provides us with a method of discerning how chlorophyll may respond over much larger distances with point data that is separated by a metre scale rather than 10s of metres.

In vivo fluorescence and patch definition

In vivo fluorescence is an extremely sensitive and specific method for describing the distribution of marine phytoplankton (Falkowski and Kolber 1995; Cullen *et al.* 1997). It is also useful because measurements can be made continually along (horizontal) transects, vertical profiles, or long deployments (Cullen *et al.* 1997). Continuous measurements also allow unambiguous interpretations that are often not possible with the more traditional methods based on sampling at discrete stations (Pingree *et al*. 1975). Using this method on directional transects around tuna cages and controls we showed that patches were evident 4 out of the 5 sampling days in the first 0–100 m of TC1, and on 3 out of the 5 sampling days at TC2 (chapter 4). The patches at the TC2 site were not evident in the chlorophyll samples so they were missed. Therefore this study establishes that low sampling resolution horizontally and statistical pooling of data is

unlikely to deliver ecologically sensitive results for monitoring programs. Patches are made up of species of phytoplankton that form aggregations in the water column at different times of the year. They may bloom together or sequentially at different times.

Chlorophyll and fluorescence signals were suppressed during the neap tide events which resulted in a homogenous effect at all sites and few patches were evident. A period of turbulent weather will cause the surface water to be mixed into the bottom layers. Patches of phytoplankton move on rapidly in turbulent environments (Denman and Gargett 1995). Depending on wind speed, current shear and stratification, time scales for cycling phytoplankton by turbulent eddies and mixing in the sea have been found to vary from about 0.5 to 100 h for vertical displacements of 10 m. The mixing response of the surface layer to the onset of strong wind is rapid, a few hours, and quickly reduces cycling times by turbulent eddies to less than 1 h (Denman and Gargett 1995). Boston Bay is a well-mixed environment with no stratification detected in this or prior studies so there are no density gradients and mixing may take place in the vertical depending on wind speed and currents. Sampling was carried out during calm weather in the mornings but in the afternoons, on-shore south easterly winds had sprung up (15 to 20 knots) on days 3 and 4, which effectively mixed the surface layer through turbulent action despite the lack of water movement caused by the neap tides.

Cages effects at fish farm sites

There is little in the literature on the physical effects of sea cages on phytoplankton communities in the surrounding waters. It was suggested in chapter 2 that the cages which slow the diffusion of water as it moves through them (Beveridge 1996) may act to enhance nutrient resuspension from sediments beneath the cages and also trap phytoplankton in the area of the cage long enough for reproduction or uptake of nutrients to take place. Additionally it is possible that the tuna cages shed fouling microalgae from their surfaces which are homogenised over the water column and create a patch at a distance from the cage. Future work might look at cage dynamics and for halo patches of shedding microalgae around the cages.

Phytoplankton distribution and abundance

Overall phytoplankton abundance showed no significant differences between tuna cages and controls; this is explained in the high variance returned between counts of phytoplankton samples. Until long-term sampling and counting of phytoplankton happens in the region it is difficult to determine any shifts in phytoplankton community structure. However, this study did give some preliminary insight into phytoplankton community structure and habitat preference in Boston Bay and Spencer Gulf (chapters 2 and 4). Picoplankton were often the most abundant organisms. Diatoms made up a high 74% of the phytoplankton community at the lower Spencer Gulf sites. Diatoms were also dominant in Boston Bay and upper Spencer Gulf but at 51%, this was a somewhat mixed community or more so than that of lower Spencer Gulf.

Dominant organisms for lower Spencer Gulf sites included *Guinardia striata*, *Chaetoceros* spp., *Nitzschia longissumum, Pseudonitzschia* spp., *Navicula* spp., *Cylindrotheca closterium, Leptocylindricus* spp., and *Licmorphia* spp., compared to Boston Bay sites where *Gymnodinium* spp., *Gyrodinium* spp., *Karenia brevis,* and *K. mikimotoi, Cylindrotheca closterium, Chaetoceros* spp., *Leptocylindricus* spp., and *Navicula* spp., were the dominant organisms. In general, for the group of *Gymnodinium* spp., the smaller dinoflagellates, such as *Heterocapsa* spp., *Torodinium* spp., and *Katodinium* spp., were most common. The diatom dominated assemblage is similar to that found in south eastern Brazil off Parana State from May to June when neritic diatoms are common (Brandini and Fernandes 1996).

The most abundant organisms were the diatoms *Pseudonitzschia* spp., *Cylindrotheca closterium, Leptocylindricus danicus* and *Chaetoceros* spp. These organisms bloomed together from autumn to mid-winter, in what appeared to be a gradual process, culminating in a major bloom in the months of June/July. The various gymnoid species also formed significant individual and community patches in Boston Bay (chapter 2 and 4). For example, the small to intermediate sized unarmoured dinoflagellates, *Karenia brevis*, *Gymnodinium* spp., a large group of small, unidentified gymnoids and *Gyrodinium* spp., bloomed together late summer and autumn, whereas *K. mikimotoi* bloomed in the cooler months. Functional groupings of these species indicate that they survive dispersal and can grow within coastal currents and frontal zones as well as nutrient rich environments (Smayda and Reynolds 2001).

Dinoflagellates were significant at Boston Bay sites and the upper Spencer Gulf site, particularly *Karenia brevis, K. mikimotoi, Gyrodinium* spp., and the smaller gymnoids, *Katodinium* spp., *Heterocapsa* spp., and *Torodinium* spp. Studies in Denmark in the Kattegatt have shown that dinoflagellates are often important in the phytoplankton community during periods of large nanoplankton biomass, particularly larger diatoms (Hansen 1991). Diatom blooms were very low during the *Karenia brevis,* and *K. mikimotoi* blooms, which may have been due to grazing by the heterotrophic dinoflagellates (Hansen 1991) as well as the ability of dinoflagellates to out-compete diatoms when conditions are optimal. Dinoflagellate significance may be a common feature in Australian temperate bay waters.

Boston Bay is a particularly well mixed environment so how do phytoplankton manage to maintain patches or aggregations in the water column? Phytoplankton species form aggregations within phytoplankton communities by either an ability to swim or through turbulent forces from water movement and an ability to uptake nutrients rapidly (Collos 1980; Cullen and Horrigan 1981; Yamamoto and Okai 2000). In areas with a rapid flushing time, it has been suggested that there is limited uptake of fish farm wastes because phytoplankton are not present long enough to capitalise on the high production of nutrients (Gowen *et al.* 1992). Experiments show that often this may not be the case and many species have mechanisms that enable them to stay in one place longer to utilise available nutrients and reproduce (Yamamoto and Okai 2000). This provides a distinct evolutionary advantage to many marine phytoplankton.

A mechanism for rapid uptake of nutrients may advantage phytoplankton as they pass through nutrient enriched areas. For example, Collos (1980) determined that if the diatom *Phaeodactylum tricornutum* is in a nitrogen limited environment for periods of between 1 and 5 days, the lag in nitrogen uptake mobilization increases from 30 to 60 minutes but for shorter periods of starvation (2-4hrs) there is no lag in mobilization of the uptake of nitrogen. Similarly, Fogg (1991) reports that nutrient-depleted algal cells may take up ammonium ions extremely rapidly and experiments in culture show that appreciable amounts can be absorbed during brief exposures to high concentrations (in Fogg 1991). This is highly species dependent.

A fish farm cage in Boston Bay is 50 m in diameter and in Boston Bay and the Southern Channel currents average from <2.5–12.2 cm/s (1.5–7.3 m/min) respectively. Nitrogenous plumes have been reported at salmon fish farm cages, which are 15 m in diameter, of at least 30 m (EAO 1996). Therefore, a nitrogenous plume at a tuna cage, which is 50 m in diameter, may conservatively extend for 100 m from the cage. In this case it would take a nonmotile diatom anywhere from 20 to 100 minutes to move through the cage/plume area and this may be sufficient time for uptake to occur. Furthermore, where there are over 60 fish farm sites, as there are east of Boston Island, there will be considerably more than one nitrogenous plume for phytoplankton to move through. Future research may determine how large these plumes are.

Karenia brevis

K. brevis cells were widely dispersed in Boston Bay and did not appear to be associated with any particular fish farm site. This is probably due to *K. brevis* motility. Cells undergo a diel vertical migration where they aggregate at the surface during the day and are able to disperse widely in the water column during the night (McKay *et al.* 2003). *K. brevis* bloomed in summer and autumn in large numbers in Boston Bay (see Figure 1) and cells appeared in lower Spencer Gulf samples in very low numbers in March 1998. Numbers were also low at the upper Spencer Gulf sites. Tester *et al.* (1993) found that variations in the distribution and abundance of *K. brevis* have been linked to changes in water temperatures and circulation as has been found in Florida waters, where cells are transported by the Gulf Loop current. Water temperatures were no different at the upper Spencer Gulf sites from sites in Boston Bay; therefore, the cells found at these sites were more likely transported from the bay through the northern channel and into the gulf.

Geesey and Tester (1993) hypothesised that the greater abundance of *K. brevis* in near-shore waters in Florida may be related to increased nutrients. Experiments have shown that *K. brevis* cells grow quite successfully on different types of nitrogen, particularly nitrate, ammonium and urea (Richardson and Pinckney 2003). Ammonia was particularly prevalent in Boston Bay during the time of the *K. brevis* blooms.

Blooms of *T. erythaeum* have been associated with the parallel development of *K. brevis* blooms (Paerl and Bebout 1988; Tester *et al.*

1993; Walsh and Steidinger 2001). It is possible that the presence of *K. brevis* in Boston Bay waters during this time was associated with the cyanobacterium bloom. Mulholland *et al.* (2003) demonstrated in field and laboratory experiments that N released from *T. erythaeum* can support the growth of *K. brevis.* Results also showed that *T. erythaeum* can fix N_2 at high rates with more than 50% of this new N released as NH_4 and DON, and that *K. brevis* has a high affinity for reduced N sources. *K. brevis* blooms were most likely influenced by water temperatures, input of nitrogen from the *T. erythaeum* bloom and also ammonia excreted from fish from the tuna farms.

Karenia mikimotoi

Generally, in 1998 cells counts of *K. mikimotoi* were lower than in 1997 on the western side of Boston Island. The lag time for *K. mikimotoi* appearing at sites east of Boston Island and upper Spencer Gulf leads to the assumption that *K. mikimotoi* cells on the western side of Boston Island were advected to upper Spencer Gulf sites and sites east of Boston Island near the tuna cages by the currents that move water out of Boston Bay through the northern channel. Takeuchi *et al.* (1995) note that the movement of *K. mikimotoi* blooms in Tanabe Bay, Japan, from the southern area to the northern area and then toward the entrance of the bay during the last stage of the bloom was consistent with a clear counterclockwise current in the bay and implied that the distribution of *K. mikimotoi* blooms is at least partly dependent upon this current. Flow is up and outwards through the north channel and there is a weak clockwise flow around Boston Island. *K. mikimotoi* cells would be likely then to

accumulate in the bay and, given the weak clockwise flow around the island, this may account for the accumulation of *K. mikimotoi* cells east and west of Boston Island around the tuna cages at the sites nearest the island.

Cell numbers for *K. mikimotoi* in Boston Bay and Spencer Gulf were very low compared to Omura Bay, Japan, where cell numbers during a major *K. mikimotoi* (formerly *Gymnodinium nagasakiense)* bloom exceeded $1x10^6$ cells/L (Ishimaru *et al.* 1989). Therefore it would appear that the growth of *K. mikimotoi* was somewhat limited in Boston Bay and surrounds. *K. mikimotoi* may have been limited by its slow growth rate due to advection and loss of cells (Honjo *et al.* 1990; Honjo 1994), salinity (Blasco *et al.* 1996; Yamaguchi and Honjo 1989) and selenium (Ishimaru *et al.* 1989). Salinities in Boston Bay and surrounds are generally stable at approximately 36–37; therefore, salinity may have been a limiting factor in the growth of this organism during this study. It is not known if the trace metal selenium occurs in Boston Bay and surrounds. Temperature may not be a limiting factor as *K. mikimotoi* growth rate is generally independent of rising or falling temperatures (Honjo *et al.* 1990).

Motile cells of *K. mikimotoi* and *K. brevis* are still present in Boston Bay and Proper Bay in very low numbers (South Australian Shellfish Quality Assurance Program 2006) although they do not appear to have achieved the growth rates evident in this study. These organisms obviously have mechanisms for maintaining themselves in the water column despite suboptimal conditions. In Gokasho Bay, Japan, most *K. mikimotoi* cells

have been observed during the day at 5–10 m, which suggests that maintaining themselves in the middle layer may be important in avoiding the frequently unfavorable conditions encountered at or near the surface (Honjo *et al.* 1990). Silke *et al.* (2005) suggest that blooms of *K. mikimotoi* are unpredictable events occurring intermittently in Irish coastal waters and that more than likely this species over-winters as a motile cell in low numbers awaiting a return to optimal conditions to bloom.

Sites in the middle of Spencer Gulf or further north from Rabbit Island sites (SG1) have not been sampled for *K. brevis* or *K. mikimotoi* cells so cells may occur in these areas but no blooms are recorded. A northward flowing current enters the Spencer Gulf from the Bight region, travelling along the westerly entrance of the gulf. The northerly current continues to flow up the gulf, until deflected at Tiparra Reef. This deflection creates a counter-current that runs along the opposing shore and merges with waters from east to west along the entrance of the Spencer Gulf (Bullock 1975; Bye and Whitehead 1975). Therefore, there is the opportunity for dispersal of these organisms into other areas and continual inoculation of Boston Bay sites with over-wintering cells.

Regional response

Fish farming interacts with the marine environment at various temporal and spatial scales (Karakassis *et al.* 2005). These maybe internal, local or regional as suggested in chapter 1. Over time there may be a significant regional increase in the total nutrient budget due to nutrient inputs from tuna farms as production grows. Long-term modeling of fish farm activities

in the Mediterranean have shown that a 1% increase in nutrient concentrations could be expected over a 20-year period from a production rate of 150,000 t (Karakassis *et al.* 2005). This estimate is considered small when contrasted with other anthropogenic sources of effluent to the Mediterranean (Karakassis *et al.* 2005). However, the potential for regional ecosystem impacts on the marine environment cannot be ignored, particularly when combined with other anthropogenic factors. Long-term regional effects from fish farm wastes include degradation of seagrass meadows (Delgado *et al.* 1999; Cancemi *et al.* 2003), impacts on macrobenthic assemblages (Boyra *et al.* 2004), proliferation of benthic microalgae on the seabed (Karakassis *et al.* 1999) and physical stress on hermatypic corals (Bongiorni *et al.* 2003).

Does this study indicate a regional response in Boston Bay? No information is available for effects from fish farming on the wider marine environment in the region, such as seagrass beds. However, there is chlorophyll data from the December months in the mid-1970s which show that chlorophyll in Proper Bay was 0.2 μg/L and at the southeast tip of Boston Island was 0.05 μg/L (Caldwell Connell Engineers 1976). In December 1996, chlorophyll ranged from 0.2 μg/L in Boston Bay to 1.76 μg/L near the tuna cages on the eastern side of Boston Island which is quite near the south east tip of Boston Island (Paxinos *et al.* 1996). Samples were taken in 1999 but only in March; however, sites from the east of Boston Island also showed some of the highest recorded chlorophyll levels for that area at >5.0 μg/L (chapter 1). Currents that occur on the eastern side of Boston Island running up Spencer Gulf are strong

(Noye 1996) so this is unexpected. This may indicate that there had been a shift in biomass response on the eastern side of Boston Island. Considerable long-term high resolution spatial and temporal sampling will determine if this is the case in future or due to some anomaly in natural variation.

Coastal eutrophication from anthropogenic sources has become a global problem (Howarth and Marino 2006). Major contributors have been from coastal urbanisation and subsequent nitrogen inputs and agricultural run-off. However, just as these practices are beginning to change, we have the added element of fish farm wastes. Nitrogen represents the largest pollution problem to many coastal waters and one of the greatest threats to the ecological functioning of these ecosystems (Nixon 1995; Howarth 2003). For cases where farms are not aggregated in the same place, this may not be an issue. For example, in Scotland where fish farming has not been found to contribute to blooms of harmful species (Smayda 2006), fish farms are distributed around the lochs. However in Boston Bay tuna farms sites are aggregated in one large aquaculture zone east of Boston Island with the capacity to hold 60 farms. Further, physiological characteristics of species of phytoplankton in relation to their growth response to nutrient inputs determine where eutrophication or harmful blooms may occur. In South Australian oligotrophic temperate waters, phytoplankton tend to be nutrient limited and therefore growth may be enhanced by additions of N particularly in the form of ammonium, whereas in Scotland phytoplankton are more light limited than nutrient limited in many of the lochs (Smayda 2006).

This study has not shown any significant differences for chlorophyll or phytoplankton abundance between tuna cages and controls but there may be some localised effects happening which are not detectable from discreet sampling techniques. Individual phytoplankton may be responding to the additional nutrient inputs. There may be a case for some regional ecosystem effects with increased production of tuna within the aquaculture zone east of Boston Island. Future long term studies on nutrient fluxes around the fish farm zone and phytoplankton response may determine these effects. This is the first time that ecologically relevant temporal and spatial scales of sampling phytoplankton around fish farms have been attempted in South Australia. We are aware that this study only provides a small window in time; however, it has shown that using continuous flow *in vivo* fluorescence we could detect persistent phytoplankton patches of a particular size, temporally and spatially, around tuna cages in an area equivalent to >1000 m^2 over a 15 day period (chapter 4).

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Appendix 1

Phytoplankton species list

Phytoplankton species list

No.	Genus/species	Type
1	*Achnanthes* sp.	Diatom
2	*Amphidinium* sp.	Dinoflagellate
3	*Amylax* spp.	Dinoflagellate
4	*Asterionella* spp.	Diatom
5	*Asterionella japonica*	Diatom
6	*Biddulphia* spp.	Diatom
7	*Biddulphia alterans*	Diatom
8	*Bacillaria* spp.	Diatom
9	*Bacteriastrum* spp.	Diatom
10	*Bacteriastrum delicatulum*	Diatom
11	*Bacteriastrum hyalinum*	Diatom
12	*Caloneis* spp.	Diatom
13	*Cerataulina* spp.	Diatom
14	*Cerataulina bergonii*	Diatom
15	*Cerataulina bicornis*	Diatom
16	*Ceratium* spp.	Dinoflagellate
17	*Ceratium fusus*	Dinoflagellate
18	*Ceratium furca*	Dinoflagellate
19	*Ceratium lineatum*	Dinoflagellate
20	*Ceratium longipes*	Dinoflagellate
21	*Chaetoceros* spp.	Diatom
22	*C. affinis*	Diatom
23	*C. coarctatus*	Diatom
24	*C. danicus*	Diatom
25	*C. decipiens*	Diatom
26	*C. teres*	Diatom
27	*C. pendulum*	Diatom
28	*Chatonella* spp.	Rhaphydophyte
29	*Chrysophyceae* spp.	Flagellate
30	*Climacosphenia* sp.	Diatom
31	*Climacodium* spp.	Diatom
32	*Coccolithophorid*	Flagellate
33	*Cocconeis* spp.	Diatom
34	*Cochlodinium* spp.	Dinoflagellate
35	*Corethron* spp.	Diatom
36	*Coscinodiscus* spp.	Diatom
37	*Coscinodiscus wailesii*	Diatom
38	*Cryptophyceae* spp.	Flagellate
39	*Cylindrotheca closterium*	Diatom
40	*Cymbella* sp.	Diatom
41	*Dactyliosolen* spp.	Diatom

42	*D. mediterraneus*	Diatom
43	*Dictyocha* spp.	Flagellate
44	*D. speculum*	Flagellate
45	*Dinophysis* spp.	Dinoflagellate
46	*D. acuta*	Dinoflagellate
47	*D. acuminata*	Dinoflagellate
48	*D. caudata*	Dinoflagellate
49	*D. fortii*	Dinoflagellate
50	*Dinobryon* spp.	Flagellate
51	*Diploneis* spp.	Diatom
52	*Eucampia* spp.	Diatom
53	*Eucampia zodiacus*	Diatom
54	*Euglena* spp.	Flagellate
55	*Eutreptiella* spp.	Flagellate
56	*Fragilaria* sp.	Diatom
57	*Gramatophora* spp.	Diatom
58	*Guinardia* spp.	Diatom
59	*Guinardia flaccida*	Diatom
60	*Guinardia striata*	Diatom
61	*Gymnodinium* spp.	Dinoflagellate
62	*Gymnodinium catenatum*	Dinoflagellate
63	*Gymnodinium falcatum*	Dinoflagellate
64	*Gymnodinium sanguineum*	Dinoflagellate
65	*Gyrodinium* spp.	Dinoflagellate
66	*Gyrosigma* spp.	Dinoflagellate
67	*Haslea* spp.	Diatom
68	*Hemiaulus* spp.	Diatom
69	*Hemiaulus hauckii*	Diatom
70	*Heterocapsa* spp.	Dinoflagellate
71	*Heterosigma* sp.	Rhaphydophyte
72	*Isthmia* sp.	Diatom
73	*Katodinium* spp.	Dinoflagellate
74	*Karenia brevis*	Dinoflagellate
75	*Karenia mikimotoi*	Dinoflagellate
76	*Lauderia* spp.	Diatom
77	*Leptocylindricus danicus*	Diatom
78	*Leptocylindricus minimus*	Diatom
79	*Licmophora* spp.	Diatom
80	*L. abreviatum*	Diatom
81	*Lingulum polyedra*	Dinoflagellate
82	*Lithodesmiun* spp.	Diatom
83	*Mastoglia* sp.	Diatom
84	*Melosira* spp.	Diatom
85	*Navicula* spp.	Diatom

86	*N.* f. *delicatula*	Diatom
87	*N. directa*	Diatom
88	*N. distans*	Diatom
89	*Nitzschia* spp.	Diatom
90	*Nitzschia bilobata*	Diatom
91	*N. longissima*	Diatom
92	*Oxytoxum* spp.	Dinoflagellate
93	*Oxyrrhis marina*	Dinoflagellate
94	*Paralia sulcata*	Diatom
95	*Pinularia* spp.	Diatom
96	*Pleurosigma* spp.	Diatom
97	*P. angulatum*	Diatom
98	*P. normanii*	Diatom
99	*Podolampas* spp.	Dinoflagellate
100	*Podolampas palmipes*	Dinoflagellate
101	*Polykrikos* spp.	Dinoflagellate
102	*Polykrikos schwarzii*	Dinoflagellate
103	*Probisca alata*	Dinoflagellate
104	*Prorocentrum* spp.	Dinoflagellate
105	*Prorocentrum gracile*	Dinoflagellate
106	*P. lima ?*	Dinoflagellate
107	*P. micans*	Dinoflagellate
108	*Protoceratium* spp.	Dinoflagellate
109	*Protoperidiunium* spp.	Dinoflagellate
110	*Pseudo-nitzschia* spp.	Diatom
111	*Pyrocystis lunula*	Dinoflagellate
112	*Rhizosolenia* spp.	Diatom
113	*R. erineis*	Diatom
114	*R. imbricata*	Diatom
115	*R. robusta*	Diatom
116	*R. setigera*	Diatom
117	*R. stolterfothii*	Diatom
118	*Scrippsiella* spp.	Dinoflagellate
119	*Stellarima* sp.	Diatom
120	*Stephanopyxis* sp. (probably *S. turris*)	Diatom
121	*Striatella unipunctata*	Diatom
122	*Surriella* spp.	Diatom
123	*Synedra* spp.	Diatom
124	*Tabularia* spp.	Diatom
125	*Thalassiosira* spp.	Diatom
126	*Thalassiothrix* spp.	Diatom
127	*Thalassionema* spp.	Diatom
128	*Thalassionema nitzschioides*	Diatom
129	*Torodinium* spp.	Dinoflagellate

130	*Triceratium* spp.	Dinoflagellate
131	*Trichodesmiun erythaeum*	Cyanobacterium

Appendix 2

A rapid Utermöhl method for estimating algal numbers

Rosemary Paxinos and James G. Mitchell

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