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Phytoplankton blooms and water quality of the Fleet lagoon, Dorset, UK, including studies of isolated toxic strains of *Alexandrium minutum* and *Prorocentrum lima*

By

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ABSTRACT

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Phytoplankton blooms and water quality of the Fleet lagoon, Dorset, UK, including studies of isolated toxic strains of *Alexandrium minutum* and *Prorocentrum lima*

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A two year survey (2000-2001) of the coastal Fleet lagoon, in Dorset, UK was performed with the aim of identifying the environmental conditions influencing the occurrence of phytoplankton blooms. Measurements included phytoplankton species abundance, biomass and identification, chlorophyll-a concentration, high performance liquid chromatography (HPLC) determined pigment concentrations, nutrients, temperature, irradiance, salinity, and data from a local meteorological station. Multivariate statistical methods (MDS and cluster analysis) were used to study temporal and spatial changes in species and environmental conditions. The quantification of biotoxins responsible for paralytic shellfish poisoning (PSP) and diarrhetic shellfish poisoning (DSP) in shellfish samples acquired from the Fleet lagoon was performed during 2001. The Abbotsbury embayment, at the brackish water end of the lagoon presented high levels of phytoplankton biomass, pigments and nutrients and may be considered eutrophic, in contrast to conditions observed in the east Fleet. Blooms of cryptophytes were observed at Abbotsbury during spring in both years and were related to new nutrient inputs that occur during winter. A bloom of the planktonic dinoflagellate *Prorocentrum micans* was observed in late summer-autumn 2000. The balance between freshwater and marine inputs to the Fleet and suitable conditions of nutrients and temperature plus longer water retention times were important determinants of the *P. micans* blooms in the west Fleet. Two toxic dinoflagellate species isolated from the Fleet lagoon were studied using laboratory cultures; the epi-benthic *Prorocentrum lima* that causes DSP and the planktonic *Alexandrium minutum*, responsible for PSP. DSP toxins were quantified using liquid chromatography linked to mass spectrometry (LC/MS) and PSP toxins using HPLC. HPLC determined pigment profiles and measurements of nitrate and phosphate concentrations in the growth media provided estimates of nutrient uptake rates. Scanning electron microscopy was used to study the morphology of 20 strains of *P. lima.* All *P.*

lima strains examined in the laboratory produced okadaic acid and dinophysistoxin-1, while *A. minutum* produced saxitoxin, gonyautoxin-2 and gonyautoxin-3. Maximum toxicity was observed during stationary growth phase in both species. *P. lima* is the most likely source of DSP toxins in shellfish from the Fleet, as other DSP producers were not observed in the Fleet.

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DECLARATION

This thesis is the result of work done wholly while under registered postgraduate candidature

GRADUATE SCHOOL OF THE SOUTHAMPTON OCEANOGRAPHY CENTRE

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GLOSSARY OF SELECTED THESIS ABBREVIATIONS

Abbreviation	Definition
	Phytoplankton Pigments
Chl-a	Chlorophyll-a
Chl-b	Chlorophyll-b
Chl-c_1+c_2	Chlorophyll-c_1+c_2
Chl-c_3	Chlorophyll-c_3
19'buta	19'-butanoyloxyfucoxanthin
19'hexa	19'-hexanoyloxyfucoxanthin
	Shellfish poisoning syndromes
PSP	Paralytic shellfish poisoning
DSP	Diarrhetic shellfish poisoning
ASP	Amnesic shellfish poisoning
NSP	Neurotoxic shellfish poisoning
CFP	Ciguatera fish poisoning
	Biotoxins
OA	Okadaic acid
DTX-1	Dinophysistoxin-1
DTX-2	Dinophysistoxin-2
DTX-4	Dinophysistoxin-4
STX	Saxitoxin
NeoSTX	Neosaxitoxin
GTX-1	Gonyautoxin-1
GTX-2	Gonyautoxin-2
GTX-3	Gonyautoxin-3
GTX-4	Gonyautoxin-4
dc-GTX-2	Decarbamoyl-gonyautoxin-2
dc-GTX-3	Decarbamoyl-gonyautoxin-3
dc-STX	Decarbamoyl-saxitoxin
YST	Yessotoxin
PTX	Pectenotoxin
	Others
HAB	Harmful algae bloom
HPLC	High performance liquid chromatography
LC/MS	Liquid chromatography coupled to mass spectrometry
Psi	Pressure per square inch
SEM	Scanning electron microscopy
ASW	Artificial sea water
FSW	Filtered sea water
PAR	Photosynthetic active radiation (between 400-700nm)
St.	Sampling station
LD_{50}	Lethal dose resulting in 50 per cent deaths

Chapter 1 - General introduction

1.1 Coastal lagoons

Coastal lagoons are defined as shallow, semi-enclosed bodies of water, lying parallel to the coastline and separated from the open sea by sandbanks, shingle, or less frequently, rocks (Subba Rao, 1981, Barnes, 1989). A shallow euphotic zone where environmental conditions are significantly modified by strong wind-driven turbulence and periodic tidal exchange are also prominent features. Coastal lagoons, which constitute about 13 % of the total world coastline, are highly productive and contribute large quantities of organic matter to the adjacent sea (Subba Rao, 1981).

Coastal lagoons experience forcing from river inputs, wind stress, tides, precipitation/evaporation balance, and surface heat balance, and may respond differently to these forcing functions. Water and salt balances, lagoon water quality, and eutrophication depend critically on lagoon circulation, salt and material dispersion, water exchange through the connection to the sea, and turnover, residence or flushing times (Kjerfve, 1993).

Europe, with its macrotidal coastline, possesses the lowest proportion of lagoonal coast of any continent, with only 5 % of its coast in this category (Cromwell, 1971). There are relatively few examples of this type of habitat in the British Isles, where coastal lagoons account for an area of only 6000 km^2 (excluding the bahias of Poole and Christchurch Harbours) (Barnes, 2000).

Lagoon environments are characterized by high rates of primary and secondary production. Primary production in lagoons may be dominated by either phytoplankton, benthic micro- and macroalgae, macrophytes, and in specific cases algal mats, or a combination of these (Knoppers, 1993) and often year round production occurs (Day and Yáñez-Arancibia, 1980). A strong link between the primary production of phytoplankton based lagoons and the flushing rate was shown by Vaulot and Frison (1986) for five Mediterranean lagoons, and between the trophic state and flushing rate of six lagoons in Brazil (Knoppers et al. 1991). Regarding the distribution and predominance of macrophytes and macroalgae, Thorne-Miller et al. (1983) cited in Knoppers (1993) suggested that this is related to the degree of enclosure of the lagoon, and tidal flushing. Poorly flushed lagoons are generally characterized by the macrophytes *Potamogeton* sp. and *Ruppia* sp. and filamentous green algae, whereas in well flushed restricted lagoons the macrophytes *Zostera* sp. and unattached green and red algae tend to be more abundant. In these cases, salinity represents a selection factor which is ultimately related to tidal exchange (Knoppers, 1993).

Phytoplankton species composition in lagoons depends to a great extent on the hydrological conditions (Subba Rao, 1981, Nixon, 1982). The species diversity index tends to be low in the proper lagoon, and increases in the direction of the open sea. The pattern of primary production is just the opposite, with a maximum in the lagoon, decreasing along a section moving offshore perpendicular to the coast (Subba Rao, 1981, Vaulot and Frisoni, 1986). Because of certain abiotic and biotic factors some of the phytoplankton species, particularly the motile flagellates and dinoflagellates, usually occur in patches on a scale of a few to several hundred meters and thus introduce both spatial and temporal heterogeneity (Subba Rao, 1981).

Lagoon environments are characterised by abundant sources of nutrients, efficient means of conservation and high rates of recycling (Day and Yáñez-Arancibia, 1980). The low salinity zone of lagoons is termed a nutrient trap as clays, present in high concentrations in riverine waters, clump upon contact with salt water. This flocculation process and slower currents allow the particles to settle, trapping nutrients (Day and Yáñez-Arancibia, 1980).

During the last few decades coastal waters have been receiving increasing quantities of industrial, agricultural, and sewage effluents through a variety of pathways. These anthropogenic inputs have altered the size and composition of the nutrient pool (Anderson et al. 2002), leading to the process of cultural eutrophication in coastal waters. Coastal lagoons are particularly susceptible to the process of cultural eutrophication, as

even in their natural unperturbed state they are among the most productive ecosystems of the world.

Phytoplankton blooms are frequently observed in coastal lagoons, due to their confined nature and high nutrient content. In the NW Mediterranean, confined waters of harbours, which are characterized for having reduced turbulence, high water residence times and high nutrient concentrations, have shown a high frequency of dinoflagellate bloom events (Vila et al. 2001a). The same characteristics are found in coastal lagoons. According to Vila et al. (2001a), denser blooms in confined waters occur probably as a particular response to the better conditions encountered in restricted areas, together with a lack of dilution. The origin of these dinoflagellate blooms can, however be the restricted water itself, in the case of some species, such as *Alexandrium minutum* that is never observed in open waters of the Catalan coast; or blooms can originate outside and are just amplified inside confined waters, such as blooms of *Alexandrium catenella* and *Gyrodinium impudicum* (Vila et al. 2001a).

Table 1.1 presents a list of phytoplankton species that have been reported to form blooms in coastal enclosed waters of lagoons and harbours. It is notable that the occurrence of dinoflagellate blooms is a common feature of such systems. Some dinoflagellates like *Prorocentrum* spp., particularly *P. micans* and *P. minimum*, *Alexandrium* spp., particularly *A. minutum* and other athecate species, e.g. *Gymnodinium* spp. are frequently observed in coastal lagoons. Bloom formation is, to a large extent, linked to the possibility of biomass accumulation in these water bodies with long residence times and low physical dispersion during periods of suitable environmental conditions for growth (Steidinger, 1983). Local hydrographic and meteorological conditions, as well as the salinity of coastal lagoons also play a role in determining the resulting dinoflagellate assemblage. Furthermore, coastal lagoons tend to be shallow, which can favour the formation and hinders dispersion of nearby dinoflagellate cyst beds (Vila et al. 2001a).

Table 1.1: Phytoplankton species that bloom in confined waters of brackish coastal lagoons and harbours.

Species found	Place	Reference
Gyrodinium resplendens	Swanpool lagoon, Cornwall, UK	Crawford et al. 1979
Alexandrium minutum	Ganzirri lagoon, Sicily, Italy	Giacobbe et al. 1996
Alexandrium catenella	Thau lagoon, France	Lilly et al. 2002
Alexandrium minutum	Harbours along the NW Mediterranean	Vila et al. 2001a, Delgado et al. 1990
Alexandrium minutum, Prorocentrum micans	Port river, Australia	Canon, 1996
Alexandrium tamarense	The hypereutrophic Bassan and Polesenon lagoons at the Po river delta, Italy	Sorokin et al. 1999
Prorocentrum micans, Gyrodinium impudicum (as *G. catenatum*)	Fusaro lagoon, Thyrrhenian Sea, Italy	Carrada et al. 1991
Diatoms (*Skeletonema costatum, Asterionela japonica, Nitzschia* sp.) during winter and dinoflagellates (*Exuviella compressa, Gymnodinium nelsoni*) and small flagellates during summer	Mediterranean coastal lagoons (Thau, Mauguio, Biguglia, Diana and Urbino) in southern France	Vaulot and Frisoni, 1986
Prorocentrum minimum, Prorocentrum micans	Sete Harbour in southern France	Grzebyk et al. 1997
Dinophysis acuminata, Dinophysis acuta	Aveiro lagoon, Portugal	Vale and Sampayo, 2003
Prorocentrum minimum, Cylindrotheca closterium	Santo André lagoon, Portugal	Macedo et al. 2001
Rhodomonas spp. and *Cryptomonas* spp. in winter, *Nitzschia closterium* during summer and *Ceratium furca* during autumn	The hypersaline Mar Menor lagoon, Spain	Gilabert, 2001
Prorocentrum maximum	Inner estuary of the Gulf of Guayaquil, Ecuador	Jimenez, 1996
Ceratium furca, Prorocentrum micans, Prorocentrum minimum, Scrippsiella trochoidea	Harbour of Alexandria, Egypt	Ismael, 2003

1.2 Harmful algal blooms (HABs)

There is a potential relationship between the apparent increase in the occurrence of harmful algal blooms (HAB) in coastal waters and the accelerated eutrophication of these areas due to human activities (Paerl, 1988, Hallegraeff, 1993, Richardson, 1997, Anderson et al. 2002). It is not clear however as to how much of this increase reflects the improving scientific awareness of toxic species and seafood quality versus an actual increase in the number, severity or frequency of harmful blooms (Anderson, 1989). Many new bloom species are believed to reflect the discovery of a hidden flora which had existed in these waters for many years without being detected (Smayda, 1989 cited in Anderson et al. 2002). The utilization of coastal waters for aquaculture and the transport of dinoflagellate cysts either in ship's ballast water or associated with the movement of shellfish stocks from one area to another may also be contributing factors to the global expansion of harmful algal blooms (Hallegraeff, 1993).

HABs can be caused by different species of microalgae, but have one unique feature in common, they cause harm, either due to the production of toxins or to the manner in which the cells' physical structure or accumulated biomass affect co-occurring organisms and alter food web dynamics (Anderson et al. 2002). HAB impacts include mass mortalities of wild and farmed fish and shellfish, illness and death of mammals, seabirds, and other animals, and alteration of marine habitats and trophic structure (Anderson et al. 2002). Moreover, human illness and death from consumption of toxic seafood or from toxic exposure through inhalation or water contact has also occurred.

A distinction must be made between two different types of HABs, those that involve toxins or harmful metabolites and can cause seafood poisoning, and those which are non toxic, but cause harm in other ways. Many HAB species that do not produce toxins are able to cause harm through the development of high biomass, causing water discoloration and leading to foams or scums, the depletion of oxygen during bloom decay, or the destruction of habitat for fish or shellfish by shading of submerged vegetation (Anderson et al. 2002). These blooms can cause indiscriminate kills of fish and invertebrates due to oxygen depletion, that occurs as a result of high respiration by the algae, or more commonly, by bacterial respiration during bloom decay. Some of the species involved are the dinoflagellates *Noctiluca scintillans*, *Scrippsiella trochoidea* and *Prorocentrum micans* (Hallegraeff, 1993). Other species can seriously damage fish gills, either mechanically or through production of haemolytic substances. Caged fish appear to be extremely vulnerable to such noxious algal blooms. Examples of these species include the diatom *Chaetoceros convolutus*, the dinoflagellate *Karenia mikimotoi* (=*Gymnodinium mikimotoi*) the prymnesiophytes *Chrysochromulina polylepsis* and *Prymnesium parvum*, and the raphidophytes *Heterosigma akashiwo* and *Chattonella antiqua* (Hallegraeff, 1993).

Only 40 or so phytoplankton species are known to have the capacity to produce potent toxins that can be accumulated in fish and shellfish and then pose some harm to humans (Hallegraeff, 1993). These toxins cause different syndromes in human consumers that are named according to the symptoms they cause as paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), neurotoxic shellfish poisoning (NSP) and ciguatera fish poisoning (CFP). Some algal toxins are extremely potent, and low density blooms can be dangerous, sometimes causing poisonings at concentrations as low as a few hundred cells per litre (Anderson et al. 2002), e.g. in the case of DSP poisoning from *Dinophysis* spp.

Not only do HABs pose a threat to public health, but they are also responsible sometimes for mass mortalities of shellfish, and they can result in great economic hardship to the coastal fishing industries and aquaculture facilities (Shumway, 1990). Estimated economic losses to the shellfish industry as a result of toxic algal blooms are abundant in the literature and shellfish monitoring programs have now been established in many countries to protect public health.

Algal toxins are secondary metabolites and as such are distinct from those involved in primary and intermediate metabolism, because they have no known key role in the basic functioning of the producing cell (Wright and Cembella, 1998). Some hypothetical roles may be intrinsic (e.g. protection from UV light, intracellular nutrient accumulation, or a differentiation signal) or extrinsic (e.g. toxic to predators, allellopathic role, a promoter of symbiotic relationships, a metal scavenger such as siderophores) (Plumley, 1997) or as plankton pheromones that promote mating of gametes (Wyatt and Jenkinson, 1997). However, for most of these metabolites, evidence of functional significance is merely speculative or supported by rather weak data (Wright and Cembella, 1998) and the ecological or evolutionary significance of these products is unclear.

1.3 Seafood poisoning syndromes

Diarrhetic shellfish poisoning (DSP)

The first known incidences of gastrointestinal illness associated with the consumption of mussels can be traced back to The Netherlands in the 1960s (Kat, 1979) Similar symptoms were described in 1976, when a mussel poisoning case occurred in northeastern Japan (Yasumoto et al. 1978). Nowadays DSP is a widespread illness, occurring throughout the Pacific, Indian and Atlantic oceans. The prominent human symptoms are gastrointestinal disorders such as diarrhoea, nausea, vomiting and abdominal pain (Yasumoto et al. 1978) with an onset time between 0.5 and 12 h after ingestion. The disease usually selfresolves within 3 days irrespective of medical treatment (Yasumoto et al. 1978) and has not caused deaths in humans.

The term diarrhetic shellfish poisoning has been associated with a number of different groups of toxic compounds (Yasumoto et al. 1989). These include the polyether compounds okadaic acid (OA), dinophysistoxin-1 (DTX-1), dinophysistoxin-2 (DTX-2), dynophysistoxin-3 (DTX-3), the macrocyclic polyether lactones pectenotoxins (PTX) and prorocentrolide, and the fused polyether yessotoxins (YTX) (Quilliam and Wright, 1995). The mechanism of action of PTX, YTX and prorocentrolide has not been fully established though they have been described as hepatotoxins and do not cause diarrhoea (Yasumoto et

al. 1989). Thus, only the compounds belonging to the OA and dinophysistoxin group should be considered as diarrhetic shellfish toxins (Aune and Yndestad, 1993, Quilliam and Wright, 1995).

The DSP toxin complex are produced by marine dinoflagellates belonging to the genera *Dinophysis* and *Prorocentrum*. Most reported DSP events have implicated *Dinophysis* spp. as the causative dinoflagellates (Bravo et al. 2001, Vale and Sampayo, 1999, Rhodes and Syhre, 1995) and the toxigenicity of *D. acuminata*, *D. acuta*, *D. fortii*, *D. mitra*, *D. norvegica*, *D. rotundata* and *D. tripes* has been confirmed (Lee et al. 1989). *D. acuminata* and *D. acuta* seem to be the main toxin producers in European waters, even though other species may be implicated as well. There is a recent realisation that the epi-benthic *Prorocentrum lima* may be the source of DSP contamination of shellfish in areas where *Dinophysis* is not present. Lawrence et al. (1998) suggested that *P. lima* is the most likely source of DSP contamination of shellfish in Nova Scotia, Canada, and in the Sanriku coast in northern Japan, *P.lima* is thought to be involved in local shellfish toxicity (Koike et al. 1998).

This group of toxins is composed of three parent compounds, OA, DTX-1 and DTX-2, the latter one an isomer of OA, plus their derivatives (Quilliam and Wright, 1995). A number of naturally occurring derivatives of the parental toxins have been observed. A series of related toxins found to be a mixture of derivatives of OA, DTX-1 and DTX-2 (originally named collectively as DTX-3) has been observed in shellfish tissues (Yasumoto et al. 1989, Marr et al. 1992). Several ester derivatives of OA, the diol esters, and water soluble, sulphated compounds, DTX-4, DTX-5a, DTX-5b and DTX-6 have already been isolated from *P. lima* (Yasumoto et al. 1989, Hu et al. 1992, Hu et al. 1995a,b, Norte et al. 1994, Suarez-Gomez et al. 2001) and *P. maculosum* (Hu et al. 1992). These compounds are less toxic analogues of OA, that are quickly enzymatically hydrolysed to OA when *P. lima* cells are disrupted (Quilliam et al. 1996). Most of the studies on the chemistry of DSP toxins have been conducted using *P. lima* cultures, as *Dinophysis* spp. cultures have not been successfully established.

OA, DTX-1 and DXT-2 exert their toxic effects by inhibiting three of the four major classes of serine or threonine protein phosphatases (PP): PP1A, PP2A and PP2B (Bialojan and Takai 1988). The relative toxicity of each of the okadaic acid analogues appears to relate to their affinity for PP (Nishiwaki et al. 1990).

Paralytic shellfish poisoning (PSP)

PSP toxins include a family of highly potent alkaloid neurotoxins with more than 20 naturally occurring analogues, including the highly potent carbamate toxins (STX, NeoSTX), gonyautoxins (GTX-1-4), the less potent N-sulfocarbamoyl group (B1, B2, C1-C4), and the decarbamoyl (dc-GTX-1-4, dc-NeoSTX, dc-STX) and deoxy-decarbamoyl derivatives (do-STX, do-GTX-2, do-GTX-3) (Cembella, 1998). These toxins bind to sodium channels, blocking ion transport and restricting signal transmission between neurons and muscles. Symptoms of PSP include paresthesia and perioral numbness, muscular weakness and in severe cases, can lead to death through paralysis of the respiratory system (Bricelj and Summway, 1998). PSP toxins are highly lethal, having an LD_{50} in mice (intraperitonially) of 10 $\mu g\ kg^{-1}$ (Oshima, 1995)

PSP is a worldwide problem (Hallegraeff, 1993). The species capable of producing these toxins include the following marine dinoflagellates, 11 *Alexandrium* species (among them, *Alexandrium tamarense*, *A. minutum*, *A. catenella*, *A. ostenfeldii*), *Gymnodinium catenatum*, primarily in temperate regions, and *Pyrodinium bahamense* var. *compressum*, in the Indo-Pacific and Central America; as well as freshwater cyanobacteria including *Aphanizomenon flos-aquae*, *Anabaena circinalis, Lyngbya wollei* and *Cylindrospermopsis raciborskii* (Sivonen and Jones, 1999). A number of isolates of the marine dinoflagellate *Alexandrium* spp. have been shown to be not toxic, and even among species that are known to contain toxigenic members, some non toxic strains are known, e.g. the *A. tamarense* from the Tamar estuary, in England (Cembella et al. 1987).

Exposure of zooplankton to PSP toxins may be lethal, produce sub-lethal effects, or cause active avoidance, yet many species of zooplankton accumulate PSP toxins and act as vectors in transferring toxins through the marine food web (Landsberg, 2002). Herring, cod, salmon and other commercial fish are sensitive to these toxins and die before toxins reach levels in their flesh that are considered dangerous to humans (Anderson, 1994). In the Bay of Fundy, USA, tons of herring have died after consumption of small planktonic snails that had eaten *Alexandrium* cells (Anderson, 1994). Mortalities of seabirds in northeastern England and in Massachusetts, USA; of humpback whales in New England, USA and of Mediterranean monk seals along the coast of western Sahara have been caused by the transfer of PSP toxins up the food web (Landsberg, 2002).

Amnesic shellfish poisoning (ASP)

ASP was first recognized in 1987 in Prince Edward Island, Canada, where it caused three deaths and 105 cases of acute human poisoning following the consumption of blue mussels. The symptoms of this syndrome include abdominal cramps, vomiting,

disorientation and memory loss (amnesia) and it is caused by the neurotoxin domoic acid (Bates et al. 1989 cited in Richardson, 1997), which is a tricarboxylic amino acid. This toxin disrupts normal neurochemical transmission in the brain. The diatom species *Pseudo-nitzschia multiseries*, *P. pseudodelicatissima*, *P. australis*, *P. seriata* and *P. delicatissima* have been implicated in ASP. Reports of domoic acid in seafood products have been mainly confined to the USA and Canada, and only insignificant concentrations have been detected in other parts of the world such as Europe, Australia, Japan and New Zealand (Hallegraeff, 1995). Domoic acid has already been associated with mortality of wildlife, including sea birds and sea lions in California (Scholin et al. 2000) and has also been found in faeces of humpback and blue whales (Lefebvre et al. 2002).

Neurotoxic shellfish poisoning (NSP)

NSP is a serious problem along the southeastern coast of north America and in the Gulf of Mexico. It is caused by blooms of *Karenia brevis* (=*Gymnodinium breve*), that can produce a group of polyether neurotoxins referred to collectively as brevetoxins. Brevetoxins bind to a site of the sodium channel and causes the opposite effect of saxitoxins. Two types of human poisoning are recorded: one causing paraesthesia, alternating hot and cold sensations, nausea, diarrhoea and ataxia, while the other form is characterized by upper respiratory distress and/or eye irritation, but no human fatalities have been caused by NSP (Richardson, 1997). In addition to causing human poisoning, brevetoxins affect marine organisms, fish kills have been frequently reported, and there have been a number of mortality events of marine invertebrates, birds, sea turtles, dolphins and manatees, particularly along the coast of Florida (Landsberg, 2002).

The raphidophyte *Chatonella* cf. *verruculosa* has recently been shown to produce brevetoxins, and brevetoxin-like compounds have also been found in *Chatonella antiqua*, *C. marina*, *Heterosigma akashiwo* and *Fibrocapsa japonica* (Landsberg, 2002). Thus far, however, there have been no documented cases of NSP caused by raphidophytes.

NSP was recorded for the first time in New Zealand in 1993, in the Bay of Plenty, and the dinoflagellate implicated in this event was tentatively identified as *K.* cf. *brevis*, but it is now being classified as a new species (Landsberg, 2002).

Ciguatera fish poisoning (CFP)

Ciguatera is a type of human food poisoning caused by the consumption of tropical fish that are contaminated with ciguatoxins. It is endemic through the Caribbean and tropical and subtropical Indo-Pacific regions (Lehane and Lewis, 2000). There are a suite

of ciguatera toxins that originate from the benthic dinoflagellate *Gambierdiscus toxicus*, which inhabits reefs in subtropical and tropical regions (Yasumoto and Murata, 1993). Ciguatoxin precursors and maitotoxin (MTX) originating from *G. toxicus* pass up the food chain to herbivorous fish and from them to carnivorous fish, that may pose a risk to human consumers (Landsberg, 2002). Maitotoxin is the most potent marine toxin known (Bomber and Aikman, 1988). Also known to be part of the tropical benthic dinoflagellate community, and possibly implicated in ciguatera are *Ostreopsis*, *Coolia* and *Prorocentrum* species, each of which produce their unique toxins and toxin derivatives (Tindall and Morton, 1998, Bomber and Aikman, 1988), but their involvement, if any, remains to be established (Lehane and Lewis, 2000). At least 400 fish species have been associated with ciguatera fish poisoning, however, aspects of the mechanisms of toxin transfer, or modification of the precursors to ciguatoxins through the food chain, and of accumulation of toxin in carnivorous fishes are still unclear (Lewis, 2001, Landsberg, 2002).

Because the ciguatoxin complex comprises multiple toxic components in fish, their effects on humans can be varied, even though only a few of the toxins have been identified or characterized from toxic fish. Symptoms usually associated with ingestion of herbivorous fish include gastrointestinal illness or neurological symptoms, whereas consumption of toxic carnivorous fish is more often associated with cardiovascular and neurological disorders (Lewis, 2001, Landsberg, 2002).

Azaspiracid poisoning (AZP)

A new group of toxins named azaspiracids was first discovered in mussels from the west coast of Ireland in 1995. They are structurally and toxicologically different from previous known toxins. The symptoms of AZP are similar to the ones caused by DSP and include nausea, vomiting, severe diarrhoea and stomach cramps. Azaspiracids have since been found in other areas of Ireland, in the Sognefjord in Norway, along the northeast coast of the UK (James et al. 2002), and more recently also in France and Spain (Magdalena et al. 2003). The species responsible for azaspiracid production is now known to be the dinoflagellate *Protoperidinium crassipes*.

1.4 Occurrence of seafood poisoning syndromes and toxic algae in the UK

PSP was detected for the first time in the UK in 1968 along the northeast coast, when 78 people were intoxicated following consumption of locally gathered mussel (*Mytilus edulis*) (Ayres and Cullum, 1978) and a large mortality of seabirds and sand eels was observed (Adams et al. 1968). Since 1968, PSP has been found in shellfish from the

northeast coast nearly every year. More recently, since 1996, when the biotoxins monitoring program was expanded to the south coast and Wales, shellfish containing PSP toxins have also been found in the Fal and Fowey estuaries on the southwest coast and in Milford Haven harbour, in south Wales (Higman and Morris, 2000). PSP toxicity is usually an annual event at these sites, although toxin levels may not exceed the action limit of 80 μg STXequiv 100 g^{-1} shellfish flesh (Higman and Morris, 2000). In 2002 PSP was detected for the first time at Salcombe in the southwest, from July through to November (CEFAS, 2003).

The organism responsible for incidents of PSP on the northeast coast has been reported to be *Alexandrium tamarense* (Robinson, 1968) and it has been generally assumed that this same species is responsible for PSP incidents that occur in other areas of the UK coast. However, *A. tamarense* strains isolated from the Tamar estuary in Plymouth and from the Weymouth inner harbour, both on the south coast, have been shown to be non toxic (Cembella et al. 1987, Higman et al. 2001b).

The first DSP outbreak associated with mussels harvested in the UK occurred in June 1997, when 49 patients presented symptoms of DSP (Scoging and Bahl, 1998). DSP positive results have principally been found in mussels from the northeast of England (Craster and Holy Island) and from the Solway Firth, on the northwest coast (Higman & Morris, 2000). On the northeast coast a DSP outbreak in 1997 coincided with densities of *Dinophysis* spp. in the water column up to 5240 cells L^{-1}. To date, this is the only correlation between DSP in shellfish from England and the species of microalgae producing the toxins. From 1996 to 1998, exceptionally high mortality rates of kittiwake birds (*Rissa tridactyla*) were recorded in colonies from the same location (Coulson and Strowger, 1999). Several aspects of the incident suggest that a phycotoxin was the probable cause of these deaths and analysis of livers taken from dead birds showed levels of OA up to 249 μg g^{-1} and DTX-1 at 783 μg g^{-1} (Howard, 1998), although the symptoms observed in birds were not consistent with DSP effects reported in humans (Coulson and Strowger, 1999). DSP has also been found in most shellfish harvesting areas of the south coast, like the Solent and the Thames estuary and during 2000 there were widespread closures of shellfisheries within these areas (Gubbins, 2002).

ASP has been found along the south and southeast coast inshore shellfisheries, and in offshore scallop sites of southwest England and in the Irish Sea (Gubbins, 2002).

Although PSP and DSP toxins are commonly found in shellfish at a number of sites around the UK coast, few studies have focused on the organisms responsible for the production of these biotoxins in England. Higman et al. (2001b) have compared the D1-

D2 region of LSU rRNA of toxic and non-toxic *Alexandrium tamarense* isolates from diverse areas in the UK and found that the isolates from Plymouth and Weymouth and the ones from Ireland (all non toxic) were assigned to the western European lineage while the ones from Scotland (all toxic) were assigned to the north American lineage. This study highlights a need to investigate the source of PSP toxins found in areas like the Fal estuary, on the south coast.

Regarding the organisms responsible for DSP toxicity in shellfish, low numbers of *Dinophysis* spp. in water samples compared with high incidence of toxicity may reflect the fact that DSP toxins are derived from the epiphytic-benthic *Prorocentrum* species (Higman et al. 2001a). However, no previous study has been undertaken in the UK describing the occurrence and toxicity of *Prorocentrum lima* and other epiphytic species of this genera that are potential DSP toxin producers.

1.5 Study area

The Fleet in Dorset is a 4700 km^2 lagoon lying inside Chesil beach, which forms the barrier between the lagoon and the sea (Fig. 1.1). It is a natural lagoonal inlet, with features of a percolation lagoon (Johnston & Gilliland, 2000). The Fleet has been described as the finest example of a lagoon of its type in the UK, and one of the more important in Europe. It is also the largest true lagoon in the British Isles and forms 80 % of British lagoonal habitat (Barnes, 2000). It contains many brackish water species of plants and animal life that are not found elsewhere in the UK. The Fleet is a UK Marine Special Area of Conservation (SAC) and its significance and conservation value has been recognised through a number of statutory designations.

The lagoon is 13 km long, varying in width between 65m at the Narrows and 900 m north of Chickerell Point (Fig. 1.1). It is connected to the English Channel at its southeastern end through Portland Harbour and is therefore tidal and saline. Freshwater enters from a few streams, which drain a catchment area of about 28 km^2, although seepage through the ground into the Fleet may drain from a wider area (Robinson, 1983). It is very shallow, water depth is generally between 0.1 and 1.2 m, deepening to 3 to 5 m in the first two kilometres between the tidal entrance at Smallmouth and the Narrows.

Dyrynda (1997) divided the lagoon into two ecologically and physically distinct areas: the lagoonal basin, with weak currents and fine sediments, and the inlet channel, with strong tidal currents and coarse sediments, both areas being particularly sheltered from wave action.

At Smallmouth and the Narrows the Fleet is marine in character, with strong tidal exchange to the sea. The coarser sections of the channel bed support a variety of assemblages of algae and sedentary invertebrates, alongside a profusion of small fish and crustaceans, particularly within the Narrows. Unusual species are common here, like the red algae *Gracilaria bursa-pastoris* and the sponge *Suberites massa* (Dyrynda, 1997).

The lagoonal section is further divided into three sub-sections according to physical and biological features. Littlesea (around Chickerell Point), which has a depth of 0 to 1.2 m, is the broadest, outermost section characterised by fine sediments with *Zostera marina* beds (eelgrass), intersected by deep fast flowing subtidal channels of up to 4 m deep (Dyrynda, 1997). The tide is strongly attenuated in this region and penetrates only weakly into the remaining 8 km of the lagoon, known as the west Fleet, which has a depth between 0 to 1.2 m (Robinson, 1983).

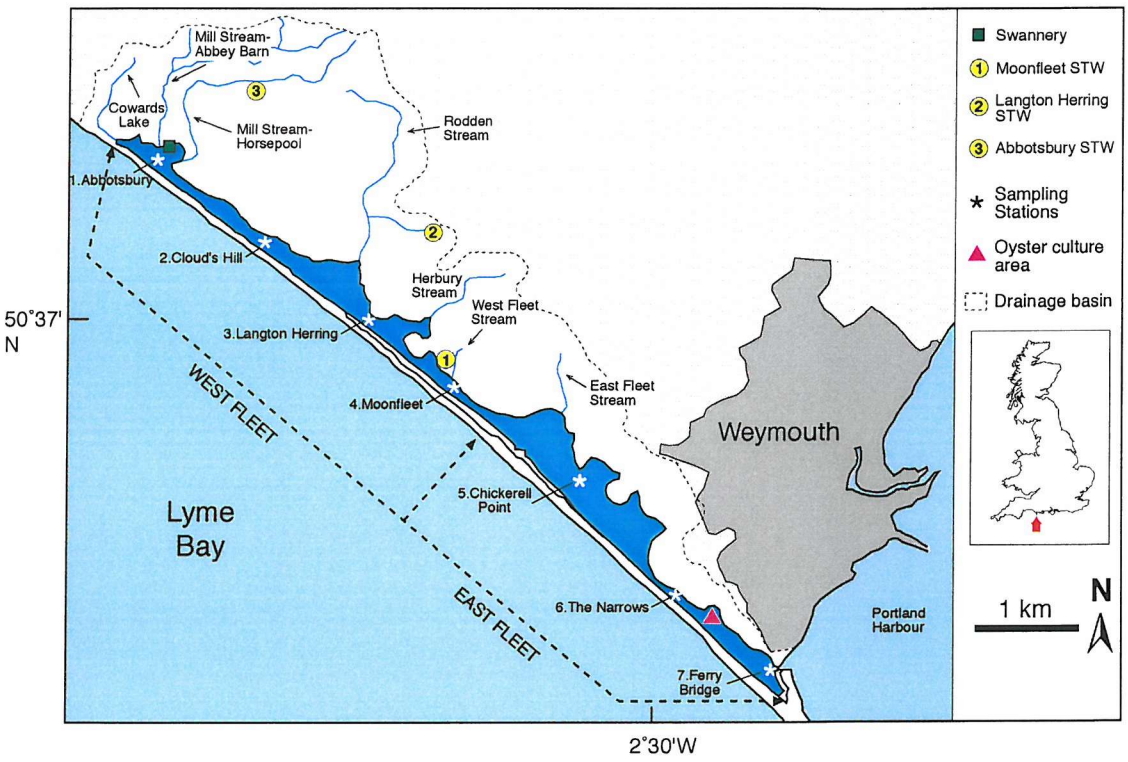


Figure 1.1: Map of the Fleet lagoon showing the drainage basin, sampling stations, the Swannery, the sewage treatment works (STW) and the location of the oyster farming.

From Moonfleet to Clouds Hill the bed of the lagoon is level and dominated by deep soft organic mud mainly colonised by seagrass meadows. *Zostera marina* is replaced towards the head of the lagoon by tasselweed *Ruppia cirrhosa*, and the rare foxtail stonewort *Lamprothamnium papulosum* is common towards the mainland shores. *Zostera noltii* and *Ruppia maritima* also occur in many parts of the lagoonal basin, and in some

areas all the species are found together (Dyrynda, 1997). The shores in this section are very narrow due to a much reduced tidal range.

The Abbotsbury embayment forms the blind head of the lagoon. Although the embayment is floored by soft organic mud, the seagrass stands are thin and patchy. The green algae *Chaetomorpha linum* is common, and in summer can be accompanied by tracts of sea lettuce *Ulva lactuca* (Dyrynda, 1997). Abbotsbury is the site of a swannery which is used by a variety of waterfowl and other aquatic birds, particularly the mute swan, and has been in existence since the 1300s (Johnston and Gilliland, 2000).

Regarding the spring neap cycle, three effects occur as an observer travels inward from the lagoon mouth; the progressive lag of high and low water, an attenuation of tidal amplitude, and a distortion of the tidal profile (Robinson et al. 1983). Indeed, during neap tides the tide is virtually non-existent in the west Fleet, when the mean water level is low, and only when the mean level is high at spring tides does it penetrate in recognisable form to Abbotsbury (Robinson et al. 1983).

The salinity structure of the lagoon is determined by the balance between tidal incursion at one end of the lagoon and freshwater inflow at the other end. The east Fleet is completely flushed in a couple of tidal cycles, irrespective of freshwater flow, while the west Fleet has water residence times varying between 10 days in high runoff and 40 days in drought conditions (Robinson, 1983).

The Pacific oyster *Crassostrea gigas* has been cultured in the Fleet since 1987 and currently oysters and mussels are grown on the sand banks close to the mouth of the lagoon (Fig. 1.1) (Copperthwaite, 2000).

Studies recently conducted by the Environment Agency (EA) have shown that the levels of some nutrients (nitrate and phosphate) are higher in the western Fleet, particularly at the Abbotsbury end (EA, 1998). Nutrients enter the Fleet through many diffuse sources. Sewage effluent from four sewage treatment works (STW) with secondary treatment is discharged into streams feeding the Fleet or directly into the Fleet itself. Concern has been expressed that the Fleet may be subjected to eutrophication, particularly the weakly flushed west region, and thus subjected to the associated potential deleterious effects on its conservation interests. However, there is little information to verify this, as historical data on water quality of the Fleet prior to 1996 is scarce. The Fleet has now been put forward by the EA as a 'candidate polluted water (eutrophic)' area to the European Community (EC) under the Nitrates Directive (Johnston & Gilliland, 2000).

Phytoplankton blooms are apparently common in the Fleet during the summer months, but there have been no previous systematic studies of the phytoplankton

community of the lagoon. Even less is known about the toxicity of phytoplankton and epi-benthic species that may be abundant in the Fleet during spring and summer. There is anecdotal information relating nutrient enrichment to summer dinoflagellate blooms in the Fleet, but the role of nitrogen and phosphorus in phytoplankton dynamics has not been systematically studied in this lagoon.

Seawater and shellfish flesh are currently sampled by CEFAS (The Centre for Environment, Fisheries & Aquaculture Science) in the Fleet and Portland Harbour to monitor for toxic algae and presence of toxins. The shellfish monitoring program was initiated in this area in 1996 and is carried out throughout the year, although more intensively during the spring and summer months. From April 1996 to March 2000 between 16 and 22 shellfish samples from the Fleet were analysed per year for the presence of PSP toxins and between 5 and 13 shellfish samples were analysed yearly for DSP toxins (Howard, 1997, 1998, 1999, 2000). These toxins were never found in shellfish from the Fleet during this time period. From April 2000 to March 2003 no PSP toxins were measured in oysters and mussels from the Fleet, although DSP was detected in oysters from the Fleet in July-August 2000 (Higman et al. 2001a, Gubbins et al. 2002, CEFAS, 2003). ASP toxins started to be systematically monitored in the Fleet in 1999, when 23 shellfish samples were analysed. Traces of domoic acid, which causes ASP were found in a mussel sample from the Fleet in November 1997 (Howard, 1998). PSP and DSP toxins are monitored by mouse bioassay according to respectively AOAC (1990) and to a modified method of Yasumoto et al. (1978), while ASP toxins are monitored using the HPLC method described by Quilliam et al. (1995).

1.6 Aims and Objectives

The overall aim of the research described in this thesis was to investigate the environmental factors influencing the occurrence of phytoplankton blooms in the Fleet lagoon and to study toxic dinoflagellate species present in the lagoon. The research involved (i) a 2 year field work component conducted in the Fleet lagoon to examine the response of phytoplankton biomass and species composition to seasonal changes in environmental variables; and (ii) a laboratory based study of the toxicity of *Alexandrium minutum* and *Prorocentrum lima* strains isolated from the lagoon.

The specific objectives of this research were as follows:

1. To relate the variation of phytoplankton biomass and taxonomic composition (species sucession) to seasonal and spatial changes in nutrient concentrations (ammonium, nitrate, phosphate and silicate), temperature, irradiance, salinity and other meteorological factors; in order to identify the environmental factors influencing the timing and duration of phytoplankton blooms in the Fleet lagoon.

2. To investigate the morphology, toxin content and pigment composition of toxic dinoflagellate species present in the Fleet lagoon. The species found in the Fleet were the DSP producer *Prorocentrum lima* and the PSP producer *Alexandrium minutum*.

3. To investigate the presence and seasonal variation of biotoxins responsible for DSP and PSP in shellfish (oysters and cockles) from the Fleet lagoon during 2001 and relate to the abundance of phytoplankton species that are potential sources of these compounds.

4. To investigate the relationship between concentrations of phytoplankton biomarker pigments and the biomass of certain phytoplankton groups observed in the Fleet lagoon.

1.7 Outline of the thesis

Chapter 1 reviews the topics of HABs and coastal lagoons, as well as the toxins produced by microalgae and the shellfish poisoning they may cause, in addition to a description of the study area and the main objectives of this research. Chapter 2 describes the methodology used during the development of the research. Chapter 3 describes the results of the field work conducted in the Fleet lagoon during 2000 and 2001, on phytoplankton composition dynamics, nutrients and other abiotic factors, as well as the presence of dinoflagellate cysts in the surface sediments of the lagoon. In this chapter multivariate analysis was used to reduce the complexity of the phytoplankton community and environmental data to allow a statistical comparison of the two detailed datasets. Chapter 4 includes a comparison between phytoplankton pigment signatures and the abundance and biomass of the major phytoplankton classes in the Fleet lagoon. Chapter 5

reports the results of toxin profile, growth rate and pigment composition of a strain of *Alexandrium minutum* isolated from the Fleet lagoon in 2000. Chapter 6 describes results of toxin profile and morphological variation of 20 strains of *Prorocentrum lima* isolated during 2000 from the Fleet lagoon. Also in this chapter results of culture experiments performed with two of these strains are described, including growth rate, toxin content and pigment composition. Finally, the main conclusions of this research are presented in chapter 7.

Chapter 2

Material and Methods

2.1 - Survey of the Fleet lagoon

Surveys of The Fleet lagoon were carried out from 18/5 to 4/11, 2000 and from 6/4 to 27/11, 2001. Sampling was undertaken at seven stations every two weeks during 2000 and at six stations at monthly intervals during 2001 (Fig.1.1). Smallmouth (station 7) was not included in the survey of 2001, as it proved to be similar to station 6 (Narrows) during the 2000 survey. Water samples were collected from just below the surface, depth samples were not collected, due to the extreme shallowness of the Fleet, which is 3-5 m deep at Smallmouth and less than 1 meter deep at low water in most parts of the west Fleet (Whittaker, 1980).

During 2000, only stations 3 and 5 were surveyed on the 18/5, while on the 1/6 only stations 1, 2 and 3 were surveyed. On 4/11 station 1 could not be sampled because the public access through the Swannery was closed. For all stations except 7, water samples were collected from the shore by wading, except for station 3 on 18/5 and 1/6, when a small boat was used for sampling. At station 7, water samples were collected with a bucket, from the Ferrybridge, the only exception was the 14/6, when the sampling was done from the shore. In 2001 the survey was initiated earlier, on 6/4/01 (as high

phytoplankton activity was observed at station 1 on 1/6/00) but only at stations 1, 2 and 6, as the access to stations 3, 4 and 5 was forbidden due to the foot and mouth disease. On 23/5/01 the access to stations 4 and 5 was still shut and these stations could not be surveyed. The sampling program was then run monthly during 2001 at stations 1 to 6 until 27/11/01. During September 2001 (from 3 to 25/9/01) a YSI 6000 multiprobe was deployed at a fixed depth approximately 100 m from the shore at station 1 (Abbotsbury embayment) to measure chlorophyll-a, temperature, salinity, pH, turbidity, dissolved oxygen and depth semi-continuously (every 10 minutes) during this time period (Appendix 1).

2.1.1 *In situ* measurements

Temperature, salinity, pH and dissolved oxygen were measured *in situ* at the surface and at the bottom of the water column with a WTW Multiline P4 universal meter and a YSI oxygen meter, model 57, with salinity correction. At station 7 (the deepest), temperature, salinity and oxygen profiles were measured at 1 m intervals throughout the water column. Probes were deployed from the Ferrybridge. Salinity was then re-measured on shore in all water samples to provide exact values. The oxygen probe was calibrated before each measurement in moist air (100 %) with the salinity compensation set to zero (freshwater) and at sea level. Dissolved oxygen was not measured at stations 1, 2 and 3 on 17/10/00 and at station 7 on the 3/10/00 due to problems with the oxygen probe. The pH meter was regularly calibrated with standard buffer solutions of pH values 7 and 10 whenever the equipment "calibration indicator" was flashing.

Total alkalinity was calculated according to a reduced volume version of the Strickland & Parsons (1972) method. A 20 mL volume sample was pipetted into a small beaker with 5 mL of 0.01 N HCl and the pH was determined using a Metler pH meter immediately after careful calibration with pH buffer 4, 7 and 10. From this measurement, total alkalinity was calculated according to Strickland & Parsons (1972). Free CO_2 (pCO_2) and total CO_2 (TCO_2) concentrations were calculated from the total alkalinity, temperature, salinity and pH measurements on each sample using a spreadsheet produced by D. Crawford. These variables were regularly measured from July to November 2000 and throughout 2001.

The light attenuation profile was measured at station 1 to 6. Light measurements were taken just above and just below the surface and at 25 cm intervals throughout the water column down to the bottom. During 2000, a Macam quantum radiometer model Q101 was used to measure the photosynthetically active radiation (PAR) (waveband 400-

700 nm). The PAR adaptor of the radiometer contains a special interference filter, which provides sharp cut-off of the near infra-red radiation and the ultra violet radiation. The adaptor is corrected to provide a 180° field-of-view. The light attenuation coefficient (k) was calculated from a linear regression of the natural logarithm of the irradiance against depth.

The euphotic layer depth, i.e. the depth where the irradiance corresponds to 1% of the surface irradiance, was calculated using the following equation:

$E_z=E_0e^{-kz}$, i.e., $(\ln E_z=\ln E_0-kz)$

If E_z corresponds to 1% light and E_0 to 100% light, z is the euphotic layer depth for a particular value of k (the light attenuation coefficient).

During 2001 a LI-1000 Licor radiometer was used, that had two sensors: one to measure the light at the surface, while the second one measures the light through the water column. k values for the 2001 dataset were calculated from plots of the natural log of values measured with the depth sensor divided by values measured with the surface sensor against depth. The slope of this regression corresponds to 1/k. The depth of the euphotic layer was calculated in the same way as for the 2000 dataset.

A total of five litres of water were collected from each station for the analysis of chlorophyll-a and accessory pigments, phytoplankton abundance and species composition, phytoplankton biotoxins, inorganic nutrients and particulate carbon and nitrogen. Net tow samples were collected with a 25 μm mesh size plankton net at stations 1, 2, 3, 4 and 6. Station 5 was too shallow and eelgrass (*Zostera* spp.) too abundant to allow deployment of the net. No preservatives were added to these net samples, which were used for microscopic observation of the live plankton community and isolation of phytoplanktonic species of interest for future culture work. Samples of eel grass and seaweed were also collected from stations 2, 3, 4, 5 and 6 with the purpose of looking for *Prorocentrum lima.*

Sediment samples from stations 1 to 6 were regularly collected using a 15 cm length of plastic tubing of 3.5 cm diameter to assess the abundance and species composition of dinoflagellate cysts from the surface sediment. These samples were kept refrigerated at 4 °C prior to analysis.

Samples of the Pacific oyster (*Crassostrea gigas*) were collected from the Abbotsbury oyster farm, close to our sampling station 7 (Fig. 1.1), during the 2001 survey for later DSP and PSP determinations. Oysters are cultivated inside metallic bags either lying on the bottom of the lagoon or over small raised racks, around 20 cm above the bottom. To evaluate if the location (bottom or rack) of the oysters would make them more likely to accumulate DSP toxins through ingestion of *P. lima* cells, oysters cultured either

on the bottom of the lagoon or on top of the racks were collected monthly and results were compared. On the 14/8/01 only oysters from the bottom were collected.

These oysters and mussels are monitored by the CEFAS laboratory for the presence of biotoxins using mousse bioassay, and biotoxins data from the year 2000 was obtained from CEFAS. Cockles (*Cerastoderma* sp.) were also collected from station 4 occasionally and later tested for DSP and PSP toxins. Shellfish were kept frozen prior to extraction and analysis of toxins.

2.1.2 Chlorophyll-a

Chlorophyll-a was determined by measurement of fluorescence, according to the method of Welschmeyer (1994). Immediately after water samples were collected a known volume of sample (usually 50 mL) was filtered using a syringe and in line 25 mm diameter GF/F (Whatman) filter. Two (during 2000) or three (during 2001) replica filters were used for each sample. The filters were kept in a cool box with ice packs during the day of sampling and were later stored in the freezer at –20 °C until further analysis. Chlorophyll-a was extracted from the filters with 90 % v/v acetone, and the extract sonicated for 30 seconds using a Vibracell sonicator. Samples were then centrifuged in a Mistral 2000 centrifuge at 3000 rpm for 10 minutes and the fluorescence measured in a Turner Designs model 10AU fluorometer fitted with a F4T41/2B2 lamp, a 436 nm excitation filter and a 680 nm emission filter. This optical combination resulted in the greatest discrimination of chlorophyll-a against chlorophyll-b, phytin-a and phytin-b providing a sensitive method for determining chlorophyll-a (Welschmeyer, 1994). 90 % acetone was used as a blank and a standard solution of chlorophyll-a (Sigma) in 90 % acetone was used to calibrate the fluorometer. Chlorophyll-a concentration in the standard solution was determined spectrophotometrically using Jeffrey and Humphrey's equation (Jeffrey and Humphrey, 1975). Absorption values at 664 nm, 647 nm and 630 nm were measured and corrected for turbidity by subtracting the reading at 750nm. A working chlorophyll-a standard solution (100 times dilution of the original stock standard) was prepared and the fluorescence measured each time a batch of samples was analysed. The fluorometer was recalibrated if the concentration of the diluted standard was more than 10 % different from the fluorometer display value. Chlorophyll-a concentrations (μg chl-a L^{-1}) were calculated according to the following equation:

$$C = \frac{f \times v}{V}$$

Where f is the average fluorescence reading, v is the volume of extract (in 90 % acetone) in mL and V is the volume of sample filtered in mL.

2.1.3 HPLC Pigments

Water samples were filtered through a 47 mm GF/F (Whatman) glass fibre filter on the day of sampling for later phytoplankton pigment analysis. Filters were kept at –70 °C prior to analysis. Pigments were extracted into 8 mL of 90 % acetone using a sonicator (Vibracell probe) for 30 seconds. The extracts were then syringe filtered through a 0.2 μm nylon filter (10 mm diameter) for high performance liquid chromatography (HPLC) analysis.

Pigments were separated by reverse phase HPLC according to Barlow et al. (1993a). A Perkin Elmer C18 column (25 cm x 46 mm i.d., 3 μm particle size) was used in the analysis of samples from the 2000 survey, while an Adsorbosphere C18 column (3 x 0.45 cm, Alltech) was used in the analysis of samples from the 2001 survey and all culture experiments. The system used was a ThermoFinigan HPLC with P2000 dual solvent pump, vacuum degasser, AS3000 autosampler, UV 6000 diode-array detector (acquired after the analysis of samples from the 2000 survey), FL3000 fluorescence detector and SN4000 system controller. All reagents used were HPLC grade.

500 μl sample aliquots were mixed with 500 μl 1M ammonium acetate and 100 μl of the mixture was injected onto the column. The mobile phase consisted of a binary eluant system with solvent A (80 % methanol and 20 % 1M ammonium acetate) and solvent B (60 % methanol and 40 % acetone). A linear gradient at a flow rate of 1 mL per minute was run from 100 % solvent A to 100 % solvent B for 10 minutes and followed by an isocratic stop at 100 % solvent B for 7.5 minutes. A second gradient of 2.5 minutes was used to return to the initial condition of 100 % solvent A. Chlorophylls and carotenoids were detected by absorbance at 440 nm, while phaeopigments were monitored with a fluorescence detector using excitation at 410 nm and emission at 670 nm. Data collection and integration utilised the Chromquest 3.0 software.

Pigment identity was secured through co-elution with authentic pigments: chlorophyll-a and -b and β-carotene were obtained from Sigma Chemical Co.; chlorophyll-c_2, chlorophyll-c_3, peridinin, 19'-butanoyloxyfucoxanthin, fucoxanthin, 19'-hexanoyloxyfucoxanthin, diadinoxanthin, diatoxanthin, violaxanthin, prasinoxanthin, alloxanthin and zeaxanthin from DHI, Denmark. Further confirmation of pigment identity was provided through on-line diode array spectroscopy.

The concentration of chlorophylls and accessory pigments in the sample, in μg L^{-1}, were determined according to the following equation:

$$C = \frac{Ap \times Ve \times 1000}{0.5 \times Rf \times Vs \times Vi}$$

Where A_p is the peak area at 440nm, *Ve* is the extract volume (in acetone 90 %) in mL, 0.5 is the buffer dilution factor, R_f is the response factor of each pigment, *Vs* is the volume of sample filtered in litres and *Vi* is the volume injected in the column (100 μl) . The R_f for the system used was previously calculated using pigment standards by Dransfeld (2000) (samples collected during 2000) or by R. Davidson (samples collected during 2001 and from all culture experiments). The *Rf* is calculated from the slope of a plot of peak area against the weight of pigment injected. The *Rf* for alloxanthin, chlorophyll-c_3 and 19'butanoyloxyfucoxanthin were not calculated by Dransfeld (2000) and the concentration of these pigments could not be calculated for samples collected during 2000.

Phaeopigments (phaeophorbides and phaeophytins) were detected by fluorescence. Concentration of phaeopigments, in μg L^{-1}, in samples collected during 2000 were calculated using the following equation:

$$C = \frac{Acp \times v \times Ri}{0.5 \times E670 \times V}$$

Where *Acp* is the fluorescence peak area at 670 nm divided by the response factor of the fluorometer, *v* is the volume of extract in mL (in 90 % acetone), *Ri* is the response factor for the UV detector, which in this case is 0.00187 for all pigments, 0.5 is the dilution factor, E670 is the extinction coefficient at 670 nm and *V* is the volume of sample in litres. For samples collected during 2001 and in all culture samples the equation used to quantify chlorophylls and carotenoids was also used for the phaeopigments. The analytical reproducibility was investigated for the 2001 dataset with two replicates of each sample, and was generally within 20.3 %.

2.1.4 Phytoplankton abundance and identification

100 mL samples from the surface of each station were preserved with 1 mL of acidic Lugol's solution for phytoplankton cell counts and taxa identification. Samples were left to settle for 24 hours in a 10 mL tubular counting chamber. Cell counts were done under a total magnification of x200 on a Leica inverted microscope. At least 200 cells were counted. Depending on cell abundance, one or two transects of the counting chamber were examined. For samples with a sparse population the whole floor of the chamber was examined and all cells were counted. Sample volumes of 5 or 1 mL were

settled to count samples of dense populations of cells. In some very dense (bloom) samples even in a 1 mL settling volume there were too many cells to count in one transect. In this case, 5 to 10 fields of view (FOV) chosen randomly were counted.

The number of cells mL^{-1} was calculated using the following equation:

$$C = \frac{Nc \times AT}{Ac \times V}$$

Where *Nc* is the number of cells counted in the area examined, *Ac* is the area examined, *AT* is the area of the whole chamber and *V* is the volume (mL) of sample settled. The value of *Ac* varied according to the magnification used.

When counting fields of view, the number of cells mL^{-1} in the sample was calculated from the equation:

$$C = \frac{Nc \times AT}{Afov \times n \times V}$$

Where *Nc* is the number of cells counted in the area examined, *Afov* is the area of one field of view (FOV), *n* is the number of FOV counted, *AT* is the area of the whole chamber and *V* is the volume of sample settled.

Phytoplankton identification was undertaken with the assistance of the following texts: Dodge (1982), Fukuyo et al. (1990), Lebour (1930), Tomas (1997) and Taylor et al. (1995).

Classification of athecate (naked) dinoflagellate species was only tentative, as important morphological features are often obliterated upon preservation and they are considered "non-preservable" species (Larsen and Sournia, 1991). Pennate diatoms were not classified to genus level, and were separated into three size classes: small (around 15 μm length), medium (around 50 μm length) and large (larger than 100 μm length).

During a visit to the laboratory of Dr. Jacob Larsen at the Botanical Institute of the University of Copenhagen, Lugol preserved samples collected from the Fleet lagoon and clonal dinoflagellate cultures were observed to confirm their taxonomic identification. Cells were observed under 600 or 1000x magnification on an Olympus BH-2 epifluorescence microscope with fluorescent illumination. Microscope slides were prepared using Lugol preserved cells and a drop of the fluorescent dye Calcofluor white (Andersen and Kristensen, 1995) to stain the thecal plates of thecate dinoflagellates.

2.1.5 Phytoplankton carbon calculation

The linear dimensions of the most abundant species in the Fleet lagoon were measured with a Leica inverted microscope at 1000x magnification using pre-settled

samples on a microscope slide. Only cells in planar view were measured and a minimum of 20 cells of each species were measured. It was shown by Hillebrand et al. (1999) that in three out of four species, the standard error is < 5 % of the mean after measurement of 20 cells. Measurements were made on cells fixed with 1 % acidic Lugol's solution. From the linear dimensions, cell biovolume was calculated according to the different geometric formulae as proposed by Hillebrand et al. (1999), except that the prolate spheroid shape was used for species that these authors suggest the use of the ellipsoid shape, e.g. naked dinoflagellates and *Scrippsiella*, as the later shape does not need the measurement of cell depth. This decision was made based on the fact that microscopy limits the measurements per cell to two dimensions, requiring assumptions to be made about the third dimension (Menden-Deuer and Lessard, 2000). Moreover, the later authors recommend the use of simple geometric formulae that require few linear measurements to estimate cell volume. Ciliate biovolume was calculated using the formulae of a sphere for *Myrionecta rubra* (=*Mesodinium rubrum*) and of a cone for *Strombidium* species.

Carbon content of each species was calculated according to the carbon to volume relationship proposed by Menden-Deuer and Lessard (2000), using equation pg C $cell^{-1}$ = 0.216 x $volume^{0.939}$ for taxonomically diverse protist plankton and equation pg C $cell^{-1}$ = 0.288 x $volume^{0.811}$ for diatoms, as dinoflagellates have been shown to be significantly more carbon dense than diatoms.

It has been shown that fixatives like Lugol's iodine can alter cell volume, resulting in shrinking or swelling. Fixation effects appear to be species specific and the magnitude and direction of cell volume change is dependent on type and strength of fixative (Montagnes et al. 1994, Menden-Deuer et al. 2001). Although some conversion equations to improve carbon content estimates from biovolume calculated from fixed cells were proposed, there are no simple approaches to correct for fixation-induced cell volume changes, particularly for estimates of mixed plankton samples containing several species. Menden-Deuer et al. (2001) concluded that fixation induced cell volume changes appear to be a negligible factor in estimates for mixed plankton. No conversion equation was used in the present work to correct for cell volume changes.

2.1.6 Dinoflagellate cysts distribution in surface sediment

The top three centimetres of each core was homogenized and a known amount of wet sediment sample (around 5 g) ressuspended in filtered seawater (FSW). Two approaches were used to screen the samples for dinoflagellate cysts. The first used primuline to stain the cellulose of the thecal plates of some species of dinoflagellates

(Yamaguchi et al. 1995) and is mainly used to look for cysts of *Alexandrium* spp., but will also stain *Protoperidinium* spp., *Scrippsiella* spp. and *Pyrophacus* sp. cysts. The second method used was a density gradient centrifugation described by Anderson et al. (1995) that uses the density of the cysts to separate them from detrital matter.

The analysis involved sonication of samples for either 2.5 minutes using a bath sonicator (for primuline stain) or for 30 seconds using a probe sonicator (for density gradient) to dislodge sediment particles. Samples were then sieved through a 255 and 125 μm mesh onto a 25 (for primuline stain) or 20 (for density gradient) μm mesh. A 20 μm mesh was used to ensure that cysts of *Alexandrium minutum* (size: 24-29 μm diameter and 15-19 μm wide, from Bolch et al. 1991) would not be lost during the sieving procedure. The sediment remaining on the 25 or 20 μm mesh was washed with FSW into a 15 mL polypropylene centrifuge tube and the volume made up to 5 mL. From this point samples were treated differently according to each method.

Samples that were primuline stained were prepared as described by (Yamaguchi et al. 1995): Sediment suspensions were fixed with 1 % glutaraldehyde for 30 minutes. After fixation, samples were centrifuged at 700x *g* for 15 minutes at room temperature and the supernatant discarded. 10 mL of methanol was added to the pellets and the tubes placed in a refrigerator for 2 days. The methanol was then replaced with 10 mL of distilled water using centrifugation. Samples were then centrifuged again, the supernatant discarded, and the pellet resuspended in 10 mL of distilled water. 1 mL of primuline solution (2 mg mL^{-1}) was added to the tubes and left for 30 minutes in the dark. After staining, supernatants containing primuline were removed using centrifugation and the pellets ressuspended in distilled water and centrifuged again for washing. The pellets were ressuspended in 5 mL of distilled water for microscope observation and 100 μl of this solution was placed in a glass Sedgwick Rafter chamber containing 900 μl of distilled water. The chamber was screened for stained cysts under a Leica epifluorescence microscope using blue excitation. Only samples collected from station 2 on 23/5/01, 14/6/01, 24/7/01, 14/8/01 and 25/9/01 were primuline stained.

A method based on density gradient centrifugation was also adopted in order to concentrate the cysts present and facilitate detection, as it removes a great amount of detritus from the sample. A "light" (density: 1 g cm^{-3}) and a "dense" (density: 1.4 g cm^{-3}) solution of the aqueous colloidal silica suspension Nalco 1060 were prepared. The light solution consists of 22.46 % (wt/vol) sucrose in distilled water, buffered to pH 8.0 with 0.05 M Tris HCl solution. The dense solution is a 88.77 %/11.23 % (wt/wt) suspension of Nalco 1060 and sucrose respectively, buffered to pH 8.1 with 0.5 M Tris HCl solution. A

step gradient was formed by placing a 5 mL cyst suspension in a 50 mL centrifuge tube. Then, 20 mL of the light solution was carefully injected underneath the sample, displacing it upwards. In the same manner, 20 mL of the dense solution was pipetted under the light solution. Tubes were then centrifuged at 3000 rpm for 30 minutes. The cysts are found at the interface between the light and dense solutions and this fraction was pipetted out carefully into a 15 mL centrifuge tube. This fraction was then washed with FSW using centrifugation and the pellets resuspended in a known volume of FSW. Variable volumes (100 to 400 μl) of this solution were placed in a glass Sedgwick Rafter chamber and the volume made up to 1 mL of FSW. Preparations were observed in a Leica inverted microscope under 100x magnification.

2.1.7 Particulate carbon and nitrogen

Water samples were filtered through GF/F (Whatman) pre-combusted (4 hours at 400 °C) 10 mm diameter filters (cut using a corkborer) in duplicate on the day of sampling. These filters were either not washed (samples from 18/5/00 to 11/7/00) or washed with 200 μl distilled water (25/7/00) or 1 mL distilled water (from 8/8/00 to 4/11/00) to elute salts. Filters were then dried for 2 hours in an oven at 60 °C and kept in a desiccator for later particulate carbon and nitrogen analysis. The analysis was performed in a Carlo Erba elemental analyser according to a modified version of Verardo et al. (1990) method. Calibration was performed using sulphanilamide as a standard, together with a pre-combusted filter, which was also used as a blank. A standard was run every 10 samples and corrections were applied when necessary. The concentration of carbon and nitrogen was calculated on a volume basis.

2.1.8 Nutrients

Water samples for nutrient analysis were syringe filtered through a 25 mm diameter GF/F (Whatman) glass fiber filter, soon after sampling. Samples for nitrate + nitrite, phosphate and silicate analyses were kept frozen at –20 °C in plastic vials for later analysis. Some samples were kept frozen for just 6 days and others for as long as 5 months. Samples collected during 2001 for silicate determinations were kept refrigerated only (not frozen). Ammonia was determined manually on samples preserved in the field, and nitrate + nitrite, phosphate and silicate were determined by segmented flow autoanalysis. Milli-Q water was used to prepare all reagents. For ammonium determinations, Milli-Q water was passed through a cation exchange resin column

previously charged with 100 mL of 10 % HCl, to eliminate any traces of ammonium. This water was used to prepare all ammonium reagents, blanks and standards.

Ammonium concentrations were measured from 14/6/00 and were determined by a spectrophotometric method, as described in Parsons et al. (1984). Filtered water samples (50 mL) were preserved in the field with 2 mL of phenol in 90 % ethanol. These phenol-preserved samples were kept refrigerated at 4 °C prior to analysis. The ammonium present in the sample reacts with phenol in alkaline citrate medium and in the presence of sodium hypochlorite and sodium nitroprusside, which acts as a catalyser. The blue indophenol colour formed with ammonium is measured spectrophotometrically. The analysis was done in four replicate sub-samples of 10 mL and the sample absorbance was measured at 640 nm in a 4 cm light path cuvette in a Hitachi U2000 spectrophotometer. The calibration coefficients for each run were always higher than 0.9991 and the analytical reproducibility was investigated with four replicates of each sample, and was generally within 6.2 %. The instrument detection limit, estimated as three times the standard deviation of the blank (Fifield, 2000) was typically 2.31 μM of ammonium.

Nitrate+nitrite, phosphate and silicate analyses were performed on an automated analytical system linked up to a digital-analysis Microstream data capture and reduction system as described by Hydes & Wright (1999). The system used was an autoanalyser from Burkard Scientific, model SFA-2, with an autosampler 80 plus connected to a chart recorder and a computer. The volume of sample used in each analysis was 2 mL and each sample was analysed in triplicate. All the methods used were colorimetric and colour density was measured using a photometer containing filters corresponding to the colour developed in the solution. The equipment was set up to measure nitrate concentrations up to 50 μM, silicate concentration up to 80 μM and phosphate concentrations up to 3 μM and have a precision of 1% at full scale. Variable working standard concentrations were prepared, according to the expected range of concentrations. A full calibration curve was run at the beginning of each batch of 64 samples and drift standards every 30 samples. NaCl 40 g L^{-1} (0.7 M) was used as the wash, blank and matrix for the working standards. Some samples needed to be diluted with NaCl (40 g L^{-1}). Drift standards of intermediary concentrations and blanks were used in the middle and at the end of each run. Peaks that were not satisfactorily separated were not considered in the calculations. New reagents were always used and the calibration coefficients for each run were always higher than 0.99991 for silicate, 0.99992 for nitrate and 0.99912 for phosphate. The analytical reproducibility of the instrument was investigated with three replicates for each sample and was generally within 1.0 % for silicate, 4.5 % for nitrate and 4.8 % for phosphate. The

instrument detection limit, estimated as three times the standard deviation of the lowest standard was typically 0.35 μM of silicate, 0.38 μM of nitrate and 0.09 μM of phosphate.

The analysis of nitrate requires the reduction of nitrate to nitrite. After passing through the cadmium column, the sample was mixed with sulphanilamide and naphthylethelynedihydrochloride (NED) to produce a pink compound with peak absorbance at 540 nm. The absorbance was measured in a 15 mm flow cell and the peaks were registered on a chart recorder. Values are presented as the sum of nitrate plus nitrite concentrations in the original sample.

Phosphate reacts with molybdate reagent in acidified medium to give a phosphomolybdate complex, which is then reduced to a highly coloured blue compound. Ascorbic acid is used as the reducing agent and antimonyl tartrate speeds up this reaction. Absorbance was measured at 880 nm in a 50 mm flow cell and the peaks were registered on a chart recorder.

Dissolved silicate reacts in acidic molybdate solutions to form yellow silicomolybdic acid, which is then reduced with ascorbic acid to form a blue compound. Oxalic acid is added prior to the reduction step to avoid phosphate interference, as this nutrient also reacts with the molybdate to form a yellow compound. Absorbance was measured at 660 nm in a 15 mm flow cell and the peaks were registered on a chart recorder.

2.1.9 Meteorological data

Meteorological data for 2000 and 2001 were obtained from the MetOffice through the BADC website (http://badc.nerc.ac.uk). Precipitation and wind speed data were downloaded from the Isle of Portland met station. Wind speed direction was initially referenced to the magnetic north. A 45° counterclockwise rotation was applied on the original data to juxtapose the axes of the wind measurements on the main and transversal axes of the lagoon. The new coordinate system has the x-axis in the direction across the lagoon, being positive towards the SW and the y-axis along the main axis of the lagoon, with the parallel wind speed component positive towards the NW into the lagoon.

Global solar irradiation was not measured at the Isle of Portland met station and was downloaded from Thorney island, the closest radiation recording met station to the Fleet lagoon. Global radiation data (W h^{-1} m^{-2}) were converted to W m^{-2} day^{-1} and then converted to photosynthetically active radiation (PAR) by multiplying for 0.45.

River Wey discharge at Broadwey, in $m^3 s^{-1}$ in a "water-day" (9:00 to 9:00 GMT) was downloaded from the following website: http://www.nwl.ac.uk/ih/nrfa/river_flow_data/ river_flow_data_download.htm.

2.1.10 Biotoxins

Most of the extractions and all the analysis of biotoxins were undertaken by me in Dr. Steven Morris laboratory at CEFAS at Burnhan on Crouch and Weymouth.

2.1.10.1 DSP toxins in seston samples

Water samples for DSP (OA and dinophysistoxins) analysis were filtered through GF/F (Whatman) filters on the day of sampling and the filters kept frozen at –20 °C. Only filters from station 5 and from the year 2000 survey were analysed. Samples from this station were chosen as some cells of *Prorocentrum lima* were observed in plankton samples collected from this location. Their presence in the water column was most likely due to the shallowness of this station and to the presence of abundant *Zostera* spp. beds, which is a substratum for the epi-benthic *P. lima*.

Extraction of DSP toxins

All solvents used for the extraction of DSP toxins from samples acquired from the Fleet lagoon were of HPLC grade. Filters retaining trapped phytoplanktonic cells were first cut into small strips and then placed into 50 mL polypropylene centrifuge tubes. Toxins were extracted from the cells using 80 % methanol in distilled water using a homogeniser (Ultra Turrax TM I-25) at 9500 rpm for 1 min according to Quilliam et al. (1996). The extracts were then centrifuged at 7500 rpm for 10 mins at 15 °C and the clear supernatants retained. The filters were extracted a second time, and the combined methanolic extracts filtered through a Whatman 0.45 μm nylon syringe filter and reduced to 200 μl using a Zymark TM TurboVap evaporator at 40 °C and 8 psi of oxygen–free nitrogen. Following the addition of 200 μl of methanol, extracts were then transferred to borosilicate vials, capped and stored at -20 °C. The homogeniser was thoroughly cleaned between each extraction with 80 % methanol in water, and on three occasions these washings were screened to ensure cross-contamination of sample extracts was controlled.

Quantitative and qualitative biotoxin analysis by LC/MS

OA, DTX-1, -2 and -4, in addition to a selection of DTX-4 diol esters were analysed by single quadrupole mass spectrometry (Platform II, Micromass, Manchester, UK) coupled to a HP 1050 High Performance Liquid Chromatograph (HPLC) (Hewlett Packard, Cheshire, UK). These analyses were performed according to the method of

Quilliam et al. (1996). A Phenomenex C8 column (150 mm x 2 mm, 5 μm) was used in conjunction with an aqueous-acetonitrile gradient containing 1mM of ammonium acetate to chromatographically separate the toxins. The flow rate was 0.2 mL min^{-1} and a four-step gradient programme was applied according to the following timetable.

Time (mins)	Acetonitrile (%)	Water + 1mM ammonium acetate
0	20	80
30	50	50
35	20	80
45	20	80

A certified reference standard of OA and an extract of the certified mussel reference standard containing OA and DTX-1 (MUS-2, NRC, Halifax, Canada) were also analysed intermittently between batches of extract samples to check the stability of the detector response and retention times of the selected OA and DTX-1 analytes. Selected ion monitoring was deployed using the deprotonated $[M-H]^-$ ions to detect OA and DTX-2 at mass to charge (m/z) ratio 803.5, DTX-1 at m/z 817.5, DTX-4 at m/z 735.3 $[M-2H]^{2-}$ and 489.9 $[M-3H]^{3-}$. The OA content in sample extracts was quantified using a certified reference calibration solution (Marine Analytical Chemistry Standards Program, National Research Council, Halifax, Canada), DTX-1 was quantified using the calibration curve generated for OA, and assuming a similar molar mass spectrometric response to OA.

2.1.10.2 DSP toxins in shellfish samples

All the Pacific oysters (*Crassostrea gigas*) analyzed during this study were acquired from the Abbotsbury oyster farm, located between our sampling stations 6 and 7. All the cockles (*Cerastoderma* sp.) analysed were collected from the bottom sediments of station 4. Prior to extraction of the DSP biotoxins, shellfish were thoroughly thawed. For oysters, the tissues of six individuals were combined, and for cockles approximately eight were pooled and thoroughly homogenised. Toxins were extracted from a sub-sample of 6 g of homogeneized tissue with 5 mL of 100 % acetone using a homogeniser (Ultra Turrax I-25) at 9500 rpm for 1 min. The extracts were centrifuged at 7500 rpm for 10 mins at 15 °C and the shellfish homogenate further extracted twice. The extracts were combined and reduced (Zymark TurboVap evaporator at 40 °C and 8 psi of oxygen–free nitrogen) to remove the acetone until an aqueous residue remained. The water volume was measured and an equal volume of ethyl acetate (EA) added to the aqueous phase to extract the toxins present in the aqueous residue. The mixture was vortex mixed for 1 min. After phase separation, the EA layer was removed by a Paster pipette, and another volume of EA was

added to the tube and vortex mixed for a further one min. Combined EA extracts were reduced to just dryness using a Zymark TurboVap evaporator (40 °C and 8 psi of oxygen–free nitrogen). Following the addition of 200 μl of methanol, extracts were stored in capped borosilicate vials at -20 °C. The homogeniser was thoroughly cleaned between samples with 80 % methanol in water. These washings were concentrated and analysed to investigate between sample cross-contamination.

OA and dinophysistoxins in shellfish extracts were quantified by LC/MS as described above.

2.1.10.3 PSP toxins in shellfish samples

Samples were thawed prior to extraction of PSP biotoxins and shellfish tissues thoroughly homogenized. 3 to 4 g of shellfish flesh were extracted with 2 mL of 0.5 N acetic acid, as described by Oshima (1995). Extracts were sonicated with a Vibra cell disruptor for 30 s while the samples remained cooled in an ice bath. Extracts were then centrifuged at 4500 rpm for 15 min in a Mistral 2000 centrifuge and stored at 4° C.

The determination of PSP biotoxins (GTX-1, -2, -3 and -4, NeoSTX and STX) was performed by HPLC with post column derivatisation following a modified method reported by Yu et al. (1998). Chromatographic separation was achieved using an Agilent 1100 Series HPLC (Agilent Technologies UK Ltd., Berkshire, UK) and a Columbus C8 guard *plus* analytical column (30 + 250 x 4.6 mm, 5 µm; Phenomenex, Cheshire, UK). The composition of the mobile phases were as follows; (A) 5.5 mM octanesulphonic acid (sodium salt) with 0.5 % tetrahydrofuran (THF) in ammonium phosphate buffer (pH 6.9 ± 0.02) and (B), 13 mM octanesulphonic acid (sodium salt) in ammonium phosphate buffer (pH 6.9 ± 0.02), 18 % acetonitrile and 1.8 % THF. The flow rate was 0.7 mL min^{-1} and a six-step gradient programme was applied according to the following timetable.

Time (min)	Eluent A (%)	Eluent B (%)
0	97	3
15	97	3
20	3	97
45	3	97
50	97	3
60	97	3

Partial chromatographic separation was found between GTX-2 and GTX-3, whereas GTX-1 and –4 were completely resolved.

A PCX5200 derivatiser (Pickering Laboratories Inc., Mountain View, CA) was employed to derivatise the post LC column effluent using an oxidation solution of 10 mM

periodic acid in 2 % ammonium solution and an acidification solution of 1 M acetic acid. The flow rates for both solutions were 0.3 mL min^{-1} and a reaction temperature of 50 °C was maintained. Fluorescence detection used excitation and emission wavelengths of 340 and 400 nm, respectively. Five point, linear calibration curves were constructed for each of the toxins following the injection of 30 µl of serially-diluted calibration solutions prepared from individual stock solutions (PSP-1C; National Research Council of Canada, Halifax, NS, Canada). These standards contained certified concentrations of STX, NeoSTX, GTX-1 and-4 and GTX-2 and -3. Calculations of specific toxicity (in pg STX equiv $cell^{-1}$) were made using Oshima's factors (Oshima, 1995).

2.2 Culture Experiments

2.2.1 Dinoflagellates isolation and establishment of cultures

Dinoflagellate species were isolated from the plankton-tow, seaweed and eelgrass samples collected from different sites along the Fleet lagoon, from May to September 2000. Macroalgae were shaken with seawater from the same site to dislodge epiphytic microalgae. These seawater samples were examined under a Leica inverted light microscope. Cultures were initiated by removing single cells from the crude samples using micropipettes and a serial dilution technique, and placing each cell into a separate well of a microtitre plate with 130 µl of K-medium (Keller et al. 1987) modified by omitting silicate, tris and ammonium and by using 308 μM of nitrate, 12 μM of phosphate, 24 μM of FeEDTA, 0.04 μM of copper and 10 μM of Na_2EDTA. This medium was prepared in Fleet GF/F filtered and autoclaved water. Cultures were later kept in sterile L-2 medium (Guillard, 1995) modified by omitting silicate, nickel, vanadium and chromium and prepared initially in Fleet GF/F filtered water and then in artificial seawater (ASW) as described by Harrison et al. (1980). All stock cultures were maintained in a temperature-controlled cabinet at 15 °C, with a light/dark cycle of 12 h: 12 h. Illumination was provided by cool-white fluorescent tubes, with a photon flux of 70 μmol m^{-2} s^{-1}. Each culture was established from a single cell and therefore is clonal (strain), but not axenic.

Two species were chosen for further investigation of their toxicity and physiology in batch culture experiments, the planktonic PSP producer *Alexandrium minutum* and the epiphytic, DSP producer *Prorocentrum lima.*

2.2.2 *Alexandrium minutum* growth experiment

Cultures used for the growth experiments were incubated in duplicate 900 mL volumes of L-2 medium (Guillard, 1995) that was modified by omitting silicate, nickel,

vanadium and chromium, and was prepared in artificial seawater (Harrison et al. 1980). Cultures were maintained in a temperature-controlled cabinet at 17 °C, with a light:dark cycle of 12 h:12 h and a photon flux density of 120 μmol m^{-2} s^{-1}. Cultures were sub-sampled on days 1, 9, 13, 17, 20 and 24.

Cell counts were performed in samples previously preserved with acidic Lugol's iodine solution using a Sedgwick Rafter chamber. The growth rate was calculated from the slope of the plot of the natural log of cell counts against culture day, during the exponential growth phase. Pigments were extracted with 90 % acetone and analysed by high performance liquid chromatography (HPLC) as described previously in section 2.1.3. In addition, chlorophyll-a was measured by fluorimetry using a Turner model 10AU fluorometer as decribed in section 2.1.2, and nitrate and phosphate concentrations determined in the culture media as described previously in section 2.1.8.

PSP biotoxins analysis in A. minutum cells

Two 30 mL of culture samples were filtered through GF/F filters (Whatman) to harvest *A. minutum* cells for toxin extraction. Filters were stored at –70 °C prior to chemical analysis. For toxin extraction, each filter was placed in a 15 mL polypropylene centrifuge tube with 2 mL of 0.5 N acetic acid. Extracts were sonicated with a Vibra cell disruptor for 30 s while the samples remained cooled in an ice bath. Extracts were then centrifuged at 4500 rpm for 15 min in a Mistral 2000 centrifuge. Extraction and centrifugation were repeated with 1 mL of 0.5 N acetic acid and the extracts were combined and stored at 4 °C.

The determination of PSP biotoxins (GTX-1, -2, -3 and -4, NeoSTX and STX) was performed by HPLC with post column derivatisation as described previously. Saxitoxin was confirmed by a single quadrupole mass spectrometer (Platform II; Micromass, Cheshire, UK) coupled to HP1050 liquid chromatograph (Agilent Technologies, Berkshire, UK). The LC was interfaced with an electrospray and was operated in positive ionisation mode. A Columbus C8 (150 x 2.0 mm; 5 µm; Phenomenex, Cheshire, UK) was used to separate the analyte and a flow rate of 0.2 mL min^{-1} of an aqueous-acetonitrile mobile phase containing 5 mM heptafluorobutryic acid was deployed over a 30 min gradient programme. The $[M+H]^+$ (m/z 300) and $[M+H-H_2O]+$ (m/z 282) ions were selected for saxitoxin monitoring.

2.2.3 *Prorocentrum lima*

2.2.3.1 Scanning electron microscopy of 20 strains of *P. lima*

Identification of *P. lima* was performed by scanning electron microscopic investigation of the surface of the thecae. Cells obtained from a mid-exponential growth phase culture were filtered through a 10 mm diameter polycarbonate membrane filter (Nuclepore) and fixed with SEM grade 4 % glutaraldehyde. Dehydration was accomplished using a graded sequence of ethanol (15, 30, 50, 70, 96 and 100 %). Filters containing the treated cells were removed, left to dry in a desicator, mounted on stubs and coated with gold. Preparations were examined on a LEO 1450VP scanning electron microscope operated at 15 kV. Cell dimensions were determined using at least 10 specimens for each clonal culture while the number of valve and marginal pores were determined using at least 5 specimens for each clone.

2.2.3.2 DSP biotoxins analysis in *P. lima* boiled wet pellets

Initial DSP screening of 20 P. lima strains

Cultures of 20 strains of *Prorocentrum lima* were successfully established in the laboratory. These cultures were kept in cotton-plugged 250 mL Erlenmeyer flasks, each containing around 100 mL of L-2 medium that was modified by omitting silicate, nickel, vanadium and chromium and prepared in filtered and autoclaved Fleet lagoon water or ASW. Cultures were kept at 15 °C, 12 h: 12 h light/dark cycle and irradiance of 70 μmol photon flux $m^{-2} s^{-1}$ for 22-55 days and were harvested by centrifugation for DSP toxin analysis.

The volume of each culture was measured and transferred to a 110 mL-centrifuge tube. A sub-sample of 3 mL was removed from each tube and fixed with acid Lugol's solution for later cell counts using a Fuchs-Rosenthal haemocytometer. Cultures were harvested by centrifugation for 20 minutes at 4500 rpm. Immediately after decanting the supernatants, the cell free medium was separated in a plastic bottle, while tubes containing cell pellets were immersed in a boiling water bath for 5 minutes to denature enzymes and preserve the native profile of biotoxins (Quilliam et al. 1996). When cell pellets are not boiled, DTX-4 is rapidly converted to diol ester upon cell disruption, followed by enzymatic conversion of diol ester to OA. The boiling technique was used to restrict enzymatic hydrolysis by heat-denaturating the enzymes involved in the biotransformations of DTX-4,-5a,-5b and -6 and diol esters into OA and allow the study of the original DSP toxin profile of *P. lima* strains from The Fleet lagoon. Culture media and cell pellets were kept frozen (-20 °C) for DSP biotoxins analysis.

Cultures of the *P. lima* strains 2.10c and 3.4g had a larger volume and two centrifuge tubes were used for each culture flask, that were treated as replicas. The culture was poured into the first centrifuge tube and an automatic pipette was used to pump the culture medium remaining in the culture flask over the bottom of the flask aiming to detach *P. lima* cells that have adhered to the bottom. The remaining volume of culture was then poured into a second centrifuge tube. The two tubes were centrifuged for 20 minutes at 4500 rpm and the culture media from each centrifuge tube was poured together and kept frozen at -20 °C. Cell pellets were treated as described previously and analysed separately.

Extraction of DSP biotoxins from boiled wet pellets

Biotoxins in boiled wet cell pellets were extracted with 1 mL of 80 % aqueous methanol using a homogeneizer (Ultra Turrax I-25) at 9500 rpm for 1 minute. The extracts were centrifuged at 7500 rpm for 5 minutes at 15 °C and cell pellets were further extracted twice. Cell pellets were left overnight in a shaker with 5 mL of 80 % methanol, to ensure complete extraction of toxins. The combined methanolic extracts were reduced to 200 μl using a Zymark Turbo Vapor evaporator (40 °C and 8 psi of oxygen–free nitrogen). Following the addition of 200 μl of methanol, extracts were stored in capped vials at -20 °C.

Extraction of OA and DTX-1 from culture media

OA and DTX-1 present in dissolved phase of the culture media (average of 120 mL of sample) were isolated by shaking with dichloromethane followed by hexane. The resulting extracts were combined and sodium sulphate was added to remove any water present. The combined extracts were then reduced to near dryness (Zymark Turbo Vapor evaporator, 40 °C and 8 psi of oxygen–free nitrogen), re-constituted in 200 μl of methanol and stored at -20 °C.

Quantitative LC/MS analysis of OA and DTX-1

The quantitative LC/MS analysis of OA and DTX-1 biotoxins in 20 strains of *P. lima* (samples used in the initial DSP screening), was performed according to Quilliam et al. (2001). The concentration of OA and DTX-1 were determined using the LC/MS equipment described above. A reversed phase column was used for the analysis (Thermo Hypersil C_8 BDS, 50 x 2.1 mm, particle size 3 μm) with a 10 mm guard cartridge of the same stationary phase. The isocratic mobile phase consisted of 2 mM ammonium formate with 50 mM formic acid in 50 % (v/v) acetonitrile. The flow rate and run time were 0.25 mL min^{-1} and 10 minutes respectively. Differing injection volumes were used depending on the sample weight equivalent in solution. The presence of DTX-4, OA and DTX-1 diol esters in cell pellet samples was assessed using the diagnostic ions described in Table 2.1.

Table 2.1: List of DTX-4, OA and DTX-1 diol esters and their $[M-H]^{1-}$, $[M-H]^{2-}$ and $[M-H]^{3-}$ diagnostic ions. Mass to charge (m/z) ratio values in bold represent selected ions used to observe the presence or absence of chosen diol esters and their parent biotoxins.

Compound	$[M-3H]^{3-}$	$[M-2H]^{2-}$	$[M-H]^{1-}$
DTX-4+SO_3+O	**521.9**	783.3	–
DTX-4+SO_3+2O	**527.7**	791.3	–
DTX-4+SO_3	**516.5**	775.3	–
DTX-4+O	**495.2**	743.3	–
DTX-4	**489.9**	**735.3**	–
OA	–	–	**803.5**
DTX-4+CH_2	494.5	**742.3**	–
DTX-4+CH_2+2O	**505.2**	758.3	–
DTX-4+2O	**500.5**	751.3	–
DTX-1	–	–	**817.5**
DTX-4+CH_2+SO_3+2O	531.9	**798.3**	–
OA diol ester	**308.5**	**463.3**	–
DTX-1 diol ester	**313.2**	**470.3**	–

2.2.3.3 *P. lima* growth experiments

The first *P. lima* growth experiment was performed using 2 replica cultures of strain 2.5a in 1L erlemmeyer flasks with 800 mL of L-2 medium prepared in ASW (Harrison et al. 1980). Cultures were kept at 17 °C and 90 µmol photon flux m^{-2} sec^{-1} (irradiance was provided by cool white fluorescent tubes) in a 12 h: 12 h light/dark cycle. Cultures were sub-sampled on days 1, 4, 7, 10, 13, 16, 22 and 28. *P. lima* cells were harvested by filtration through GF/F Whatman filters for HPLC pigments and chlorophyll-a analysis and by centrifugation for 20 minutes at 4500 rpm for DSP biotoxins analysis. Cell pellets were boiled and treated as described above.

Cell counts were performed in samples previously preserved with acidic Lugol's iodine solution using a Sedgwick Rafter chamber. The growth rate was calculated from the slope of the plot of the natural log of cell counts against culture day, during the exponential growth phase. Pigments were extracted with 90 % acetone and analysed by high performance liquid chromatography (HPLC) as described previously in section 2.1.3. In addition, chlorophyll-a was measured by fluorimetry using a Turner model 10AU fluorometer as decribed in section 2.1.2, and nitrate and phosphate concentrations determined in the culture media as described previously in section 2.1.8.

DSP biotoxins were extracted from boiled wet pellet samples from the growth experiment with 1 mL of 80 % aqueous methanol using a sonicater (Vibracell probe) for 30 seconds. Samples were then centrifuged in a Mistral 2000 centrifuge at 3000 rpm for

10 minutes and cell pellets were further extracted twice. The combined methanolic extracts were reduced to near dryness using a Zymark Turbo Vapor evaporator (40 °C and 8 psi of oxygen–free nitrogen) and ressuspended in 200 μl of methanol. Extracts were stored in capped vials at -20 °C.

LC/MS quantification of OA and DTX-1 in samples from the growth experiments were performed according to Quilliam et al. (1996). A Phenomenex C8 column (150 mm x 2 mm, 5 μm) was used in conjunction with an aqueous-acetonitrile gradient containing 1mM of ammonium acetate to chromatographically separate the toxins. The flow rate was 0.2 mL min^{-1} and a four-step gradient programme was applied according to the following timetable.

Time (mins)	Acetonitrile (%)	Water + 1mM ammonium acetate
0	20	80
30	50	50
35	20	80
45	20	80

Selected ion monitoring was employed using the deprotonated $[M-H]^-$ ions to detect OA and DTX-2 at mass to charge (m/z) ratio 803.5 and DTX-1 at m/z 817.5. The OA content in sample extracts was quantified using a certified reference calibration solution (Marine Analytical Chemistry Standards Program, National Research Council, Halifax, Canada), and DTX-1 was quantified using the calibration curve generated for OA, assuming a similar molar mass spectrometric response to OA.

However, cultures showed very poor growth rate, of only 0.022 day^{-1} during the first experiment, well below values between 0.06 and 0.47 day^{-1} reported in the literature for *P. lima* (see Table 6.10). Cell numbers were also quite variable from one sampling day to the next. This was most likely due to the sensitivity of dinoflagellate species to turbulence disturbance caused by the stirring (homogenisation) of the culture before each sampling. Growth inhibition caused by shaking of cultures has been observed in several dinoflagellate species including *P. micans* and *P. triestinum* (Estrada and Berdalet, 1998). It is possible that epiphytic species are even more sensitive to turbulent mixing than planktonic ones.

To avoid this problem, a different experimental design was used in a second experiment, when strain 2.5a was grown in 12, 250 mL flasks with 130 mL of L-2 medium in ASW under the same light and temperature conditions. This time, two flasks randomly chosen were sacrificed on days 1, 6, 12, 18, 24 and 30 while other flasks were not touched. Changes in the same parameters were followed. The growth rate however

was shown to be similar to the first experiment (0.022 day^{-1}), although higher cell densities were achieved.

During the third *P. lima* growth experiment, cultures of strain 2.9a were kept for 45 days on a longer light period, of 16 hours, as used by other authors to grow this species. 14, 250 mL flasks with 130 mL of L-2 medium in ASW were incubated at 17 °C and 90 µmol photon flux m^{-2} sec^{-1} in a 16 h: 8 h light/dark cycle. The *in vivo* fluorescence of all flasks was checked in a Turner 10AU fluorometer on the first day of the experiment to confirm all contained similar levels of biomass. Two flasks were sacrificed on days 1, 8, 17, 25, 31, 35 and 45 for the same determinations as in experiment 1. A higher growth rate was finally observed in these cultures (0.11 day^{-1}).

The analyses of samples collected during the second and third experiment were performed as described above for samples from the first experiment. Although samples of culture media were collected and kept frozen for extracellular (dissolved) OA and DTX-1 biotoxins analysis during the three growth experiments, these samples were not analysed due to time constraints.

2.3 Data analysis

Multivariate methods

Two multivariate methods, hierarchical clustering (cluster) and non-metric multidimensional scaling (MDS) were employed in this study to attempt to reduce the complexity of the high dimensional phytoplankton community and environmental datasets by taking a particular (low dimensional) view of the structure it exhibits (Clarke and Warwick, 1994). The computer software package PRIMER (Plymouth Routines In Multivariate Ecological Research) version 5.1.1, developed at the Plymouth Marine Laboratory was used to perform these analysis following the recommendations of Clarke and Warwick (1994).

Phytoplankton species data

The cluster analysis and the MDS start from a triangular matrix of similarity coefficients computed between every pair of samples. The coefficient of similarity is a measure of how close (similar) two samples are. The Bray-Curtis coefficient was used for the phytoplankton species data, as it is a satisfactory coefficient for biological data on community structure (Clarke and Warwick, 1994). The Bray-Curtis coefficient reflects differences between two samples due both to differing community composition and/or differing total abundance. "Joint absences" are ignored, as species data normally have large blocks of zero counts that need to be treated in a special way. Species accounting for

more than 3 % of the total abundance in any one sample were retained whereas rare species were excluded.

Data transformation in community analysis is used to weight the contributions of common and rare species in the (non-parametric) multivariate representations. It is acknowledged that the choice of transformation to use can have a significant effect on the final ordination or clustering display. They provide a continuum of effects from y^{λ}, where $\lambda = 1$ (no transform), for which only the common species contribute to the similarity, through $\lambda = 0.5$ (square root), which allows the intermediate abundant species to play a part, $\lambda = 0.25$ (4^{th} root), which takes some account also of rarer species, to $\lambda \rightarrow 0$, that can be thought of as equivalent to the loge(y) (or log(1+y)) transformation and would be more severe than the 4^{th} root transform (Clarke and Warwick, 1994). Alternatively, reduction to presence/absence may be thought of as the ultimate in severe transformation of counts, and will have the effect of giving equal weight to all species, whether rare or abundant (Clarke and Warwick, 1994).

The Fleet data were double root (4^{th} root) transformed to down-weight the importance of the very abundant species, so that the less dominant species contribute something to the definition of similarity, whilst the retention of some information on the prevalence of a species ensures that the commoner species are generally given greater weight than the rare ones.

The cluster analysis aims to find "natural groupings" of samples such that samples within a group are more similar to each other, than samples in different groups. It is appropriate for delineating groups with distinct community structure, with different characteristic patterns of abundance found consistently in different groups (Clarke and Warwick, 1994). The result of a cluster analysis is represented by a dendogram, with the x axis representing the full set of samples and the y axis defining a similarity level at which two samples or groups are considered to have fused.

For two different sample groups identified as the result of a cluster analysis, the species that primarily accounted for the observed assemblage difference were identified by a decomposition of the Bray-Curtis similarity into contributions from each species. The overall percentage contribution each species makes to the average dissimilarity between two groups is established and the species are then listed in decreasing order of their importance in discriminating the two sets of samples. This was accomplished by using the SIMPER (similarity percentages) routine in PRIMER.

The purpose of MDS (Kruskal's non-metric procedure) is to construct a "map" or configuration of the samples, in a specified number of dimensions, which attempts to

satisfy all the conditions imposed by the rank similarity matrix (Clarke and Warwick, 1994). MDS plots can be arbitrarily scaled, located, rotated or inverted as only relative distances between samples can be interpreted. For large data sets, it will usually not be possible to place all points in two dimensions in such a way as to satisfy the similarity ranking exactly, and there will be some distortion or stress between the similarity rankings and the corresponding distance rankings in the ordination plot. The MDS algorithm chooses a configuration of points which minimises this degree of stress. Moreover, the combination of clustering and ordination analysis can be a very effective way of checking the adequacy and mutual consistency of both representations (Clarke and Warwick, 1994). The strength of the ordination is in displaying a gradation of community composition across a set of samples.

Environmental variables

Salinity, temperature, pH, phosphate, silicate, chlorophyll-a, particulate organic carbon (POC), particulate organic nitrogen (PON), total rainfall in the two weeks preceding the sampling date and average PAR in the week before the sampling were used as variables in the multivariate analysis. POC and PON were not analysed during the 2001 survey, and therefore were not available for the multivariate analysis of the 2001 data. Other variables such as ammonium, nitrate, dissolved oxygen, total alkalinity and total CO_2 needed to be excluded from the analysis due to the large number of missing values, which would impend the multivariate statistical analysis.

There are important differences between environmental variables and species (biological) data. Abiotic data are usually on mixed measurement scales and the Bray-Curtis coefficient, that assumes a common measurement scale is not appropriate. Instead, each variable needs to be first normalised (subtract the mean, divide by the standard deviation) to put them on a common, dimensionless measurement scale, and standard Euclidean distance is then used (Clarke and Warwick, 1994).

Normalisation operates most effectively when the data are near as possible to (multivariate) normality. Pairwise scatter plots of the environmental data (Draftsman plot) should show roughly linear relationships and a symmetric distribution of points. These graphs were used to aid in choosing which transformation to use. Different transformations may be needed for different variables, but in general it is desirable to use a common form of transformation for a variable of a particular type (Clarke and Warwick, 1994). Environmental variables of the Fleet data were log transformed (ln (0.1+value)) based on examination of the Draftsman plots.

Cluster and MDS analyses were performed using log transformed data and normalised Euclidean distances, that are more appropriate for this kind of data. The matching of biotic to environmental patterns was done using the BIOENV routine of PRIMER. This routine calculates a measure of agreement between two similarity matrices, the fixed Bray-Curtis similarity biotic matrix and each of the possible normalised Euclidean distance abiotic matrices. Combinations of environmental variables are considered at steadily increasing levels of complexity, i.e., k environmental variables at a time (k = 1,2,3,...). This is done by rank correlating the matching elements in the two similarity matrices using a standard Spearman rank correlation. Their ranks can be compared through a rank correlation coefficient, as a successful MDS is a function only of the similarity ranks. Rank correlations (ρ) are computed for all possible combinations of abiotic variables.

The intuitive premise adopted in the BIOENV test is that if the suite of environmental variables responsible for structuring the community was known, then samples having rather similar values for these variables would be expected to have rather similar species composition, and an ordination based on this abiotic information would group sites in the same way as for the biotic plot. If key environmental variables are omitted, the match between the two plots will deteriorate. The match will also worsen if abiotic data which are irrelevant to the community structure are included. Following these premises, the variables chlorophyll-a, particulate organic carbon and nitrogen were excluded from the analysis, as they are closely related to the phytoplankton itself, and not determinants of the community structure.

Chapter 3

The water quality and phytoplankton community of the Fleet lagoon

3.1 Introduction

The Fleet is probably the most studied coastal lagoon in the UK. However, little is known about its water quality and plankton communities. Studies conducted by the Environment Agency (EA) have shown that the levels of some nutrients (nitrate, ammonium and phosphate) are higher in the western Fleet, particularly in the Abbotsbury embayment (EA, 1998). Concern has been expressed that the Fleet may be subjected to eutrophication, however, there is little information to verify this, as there is very little historical data on water quality of the Fleet prior to 1996.

Algal blooms have been recorded at Abbotsbury on a number of occasions since 1969 (Whittaker, 1980, Saunders-Davies, 1993, John, 1995, Jamieson, 1998), mostly during warm dry summers. Blooms in 1969 and 1976 were associated with fish deaths, and possibly with farm pollution incidents, but it is not known which species of plankton were involved on these occasions (Johnston & Gilliland, 2000). John (1995) conducted a two month summer survey of phytoplankton and nutrients along the Fleet and reported that the site near the Swannery consisted mainly of dinoflagellate species during the summer of 1995. Two dinoflagellate blooms were described, one composed of the heterotrophic species *Oxyrrhis* sp. and the other of the phototrophic *Glenodinium*

foliaceum. According to Dyrynda (1997) the water is generally clear from spring to autumn, but is temporally discoloured by intense green blooms in spring and by short lived but often intense red/brown dinoflagellate blooms within the Abbotsbury embayment in summer. Blooms of *Alexandrium tamarense* were recorded in 1996 and 1998 and a bloom of *Prorocentrum micans* was observed in 1997 (Jamieson, 1998). It is not clear if these algal blooms that have previously been reported were natural events or caused by anthropogenic inputs, and there is some concern that eutrophication of the lagoon is causing an increase in the frequency and duration of these blooms (Johnston and Gilliland, 2000).

3.2 Results

A map of the lagoon showing the location of the sampling stations is presented in chapter 1 (Section 1.5, Fig. 1.1).

3.2.1 *In situ* parameters

Salinity

There is considerable scatter in the salinity data from the Fleet, with the expected trend ranging from full sea water salinity at Smallmouth (station 7) to brackish water at Abbotsbury (station 1) (Fig. 3.1). Wider variation in salinity was observed at station 1, where values ranged from 8.1 to 30.0 (Fig. 3.2). At Smallmouth, which links the lagoon with Portland Harbour, salinity values varied from 33.8 to 35.9 (Fig. 3.2). During both years (2000 and 2001) the seasonal pattern of lower salinities during the winter and higher salinities during summer was observed in the west Fleet, and was more pronounced at the brackish water end of the lagoon (station 1) (Fig. 3.1). These higher salinity values during the summer months are related to lower freshwater input through rainfall, runoff and river discharge, plus increase in evaporation. In 2001, when the survey was started earlier than in 2000, salinity at station 1 was as low as 8.1 on 6/4/01, while salinity at this station was 12.8 on 1/6/00, the first date station 1 was visited in 2000.

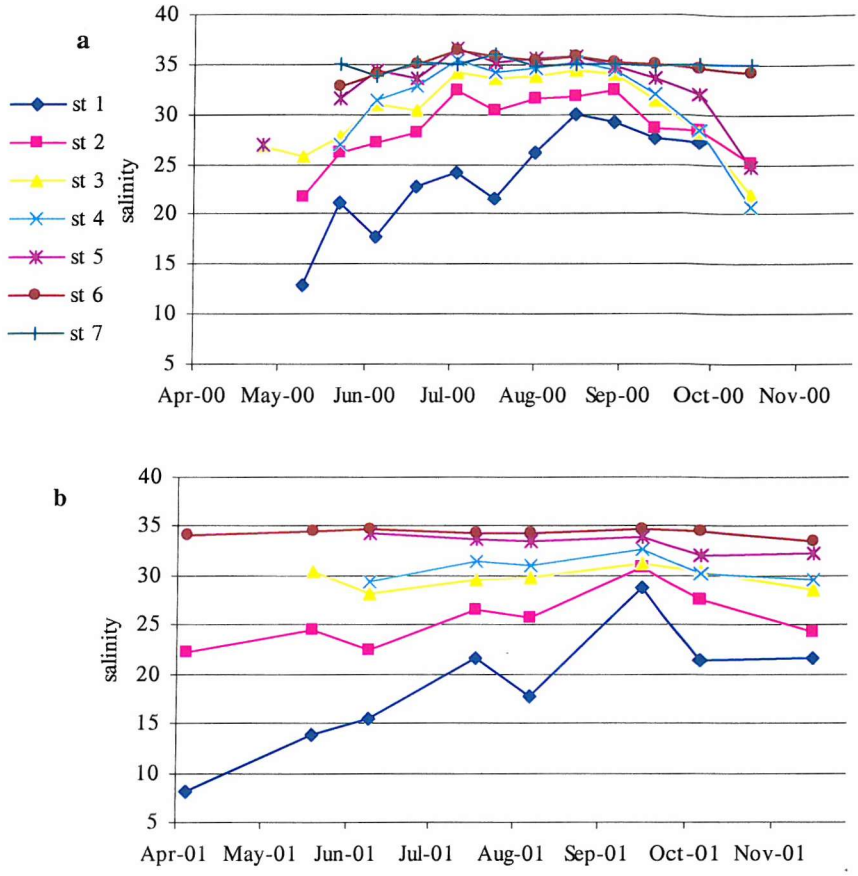


Figure 3.1: Salinity variation along the Fleet lagoon during 2000 (a) and 2001 (b).

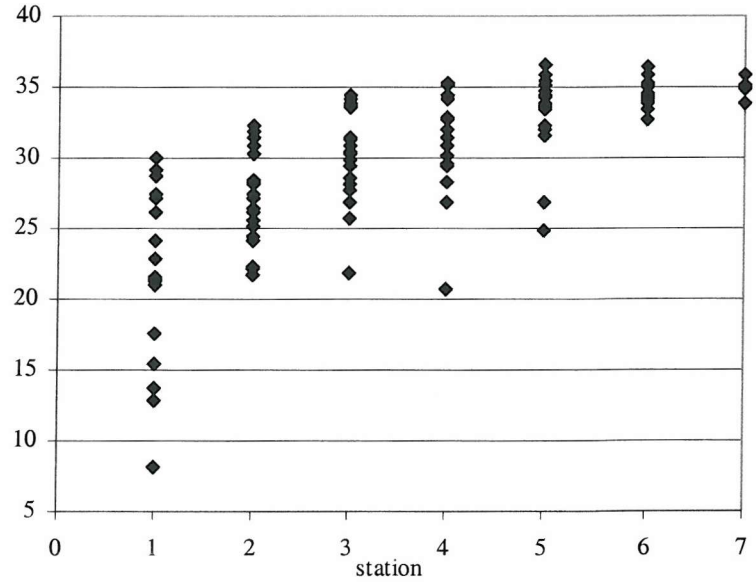


Figure 3.2: Salinity observations along the Fleet lagoon during 2000 and 2001.

Temperature

A predictable seasonal pattern of water temperature was observed in the Fleet, with higher values measured between June and August and lower temperatures in April and

November (Fig. 3.3). Maximum temperature recorded was 24.6 °C on 24/7/01 and minimum temperature was 7.9 °C on 4/11/00. Lower temperature values were observed at stations 6 and 7, the more saline stations. The temperature amplitude between station 1 and 7 varied between 0.7 °C on 1/6/00 and 7.5 °C on 23/5/01, and was on average 3.2 °C. Thermal homogeneity of the water column was observed throughout the year due to the shallowness of the lagoon (max. depth 1.2 m at stations 1 to 5) and the effects of wind mixing and tidal currents (the latter mostly affecting the eastern end of the Fleet).

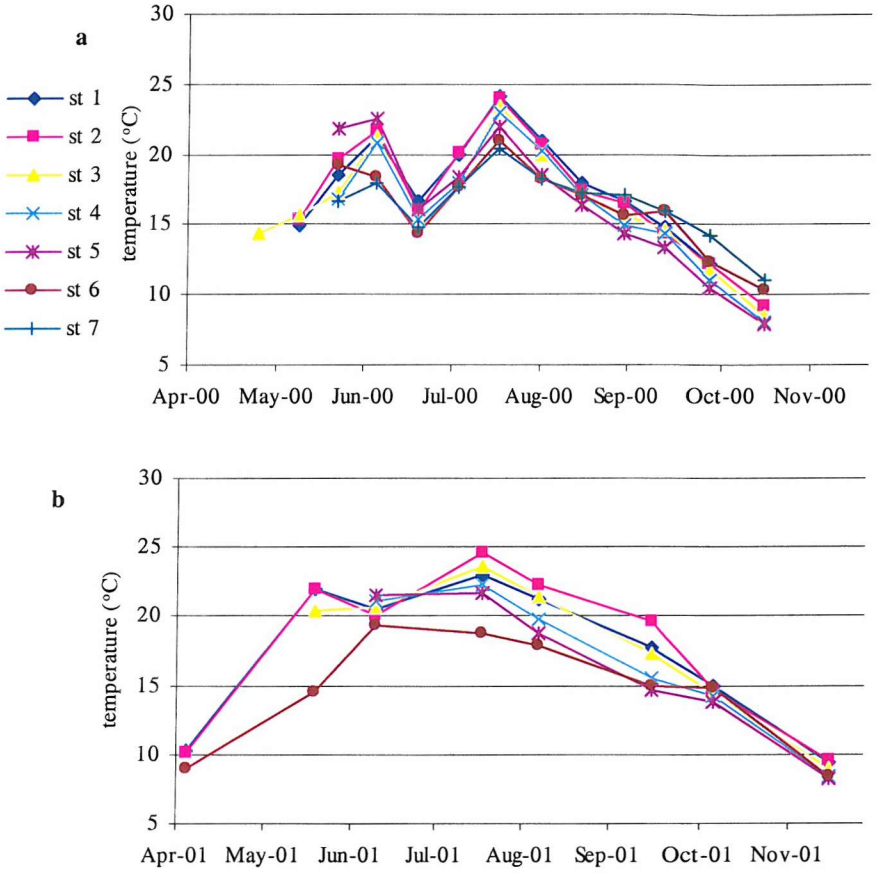


Figure 3.3: Temperature (°C) variation along the Fleet lagoon during 2000 (a) and 2001 (b).

Dissolved oxygen

Dissolved oxygen was not measured on 14/8/01 and 16/10/01 due to problems with the sensor. Dissolved oxygen values varied between 5.2 – 17.0 mg L^{-1}, 72 – 229 % saturation during 2000 and 6.2 - 14.8 mg L^{-1}, 82.8 - 194.3 % saturation during 2001. Higher dissolved oxygen values were found in the western part of the lagoon (Fig. 3.4). At stations 1 to 5 oxygen values were generally above 100 % saturation, while at stations 6 and 7 it was more often lower than 100 % saturation (Fig. 3.5).

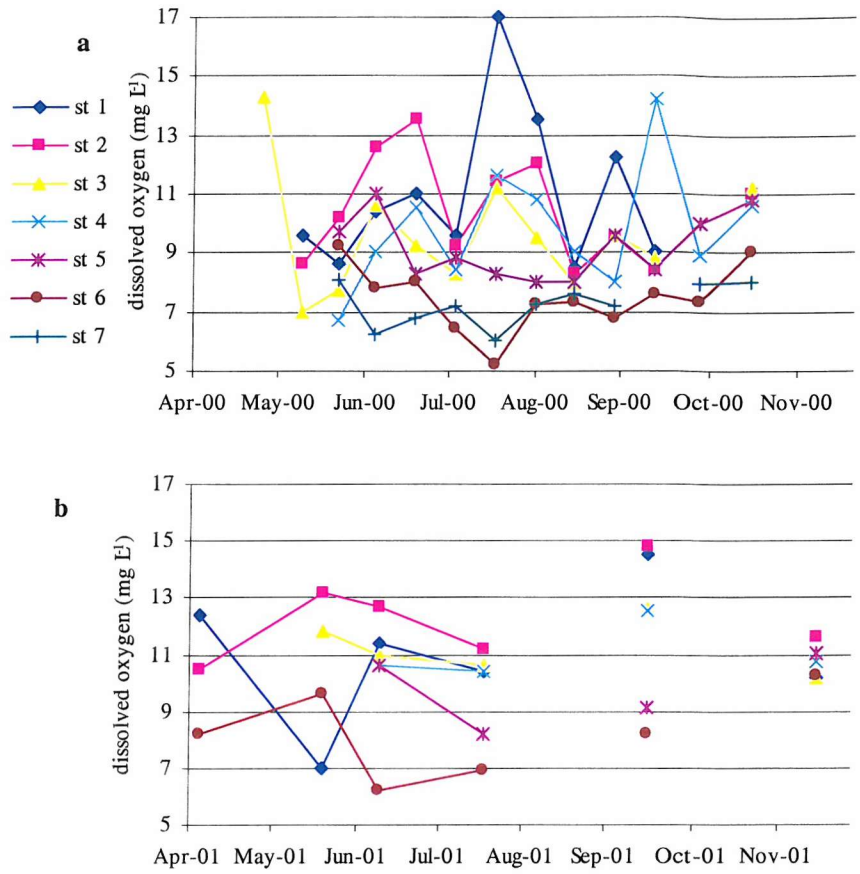


Figure 3.4: Dissolved oxygen (mg L^{-1}) variation along the Fleet lagoon during 2000 (a) and 2001 (b).

Higher oxygen values observed at stations 1 to 4 were probably related to increased rates of primary production of phytoplankton, seaweeds and eelgrass (*Zostera* spp.). A peak value of 17 mg L^{-1} (229 % saturation) was observed on 8/8/00 at station 1, when *P. micans* was growing. A high oxygen saturation value of 149 % was also recorded at station 1 on 19/09/00, and coincided with the peak in chlorophyll-a, again due to presence of *P. micans*. During 2001, dissolved oxygen values at stations 1 to 4 were often higher than 10 mg L^{-1}.

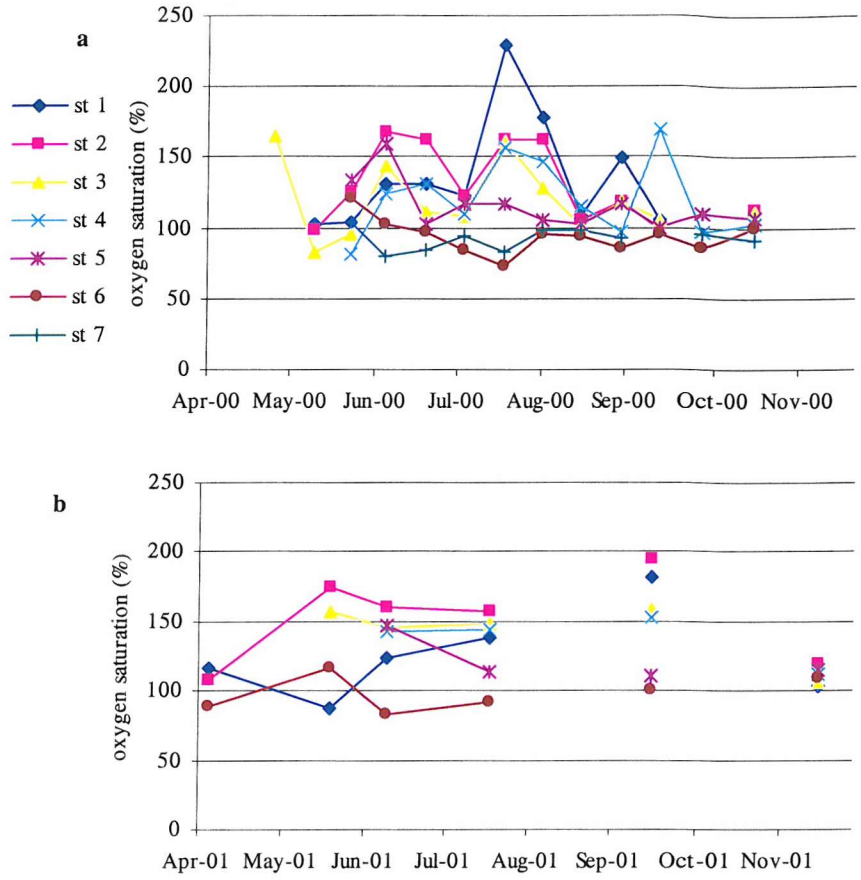


Figure 3.5: Oxygen saturation (%) variation along the Fleet lagoon during 2000 (a) and 2001 (b).

pH

During both years, pH values were higher in the mid Fleet (stations 3 and 4) and decreased towards the east and west ends (Fig. 3.6). Mean pH at stations 3 and 4 were respectively 9.14 (2000) and 9.29 (2001) and 9.06 (2000) and 9.22 (2001), while at stations 1 and 6 the average pH was 8.81 (2000) and 8.63 (2001) and 8.60 (2000) and 8.42 (2001) respectively. Station 7, at the mouth of the lagoon exhibited the lowest pH values (2000 mean 8.09). The higher pH values observed in the mid Fleet between stations 2 to 5 corresponded to the region where *Zostera* beds and seaweeds were more abundant. In June during both years extremely high pH values of slightly over 10.0 were recorded at stations 3 and 4 where the macroalgae *Cladophora* sp. was noted to be very abundant, although the suspended chlorophyll-a concentration was less than 1 $\mu g\ L^{-1}$ during this month at these stations in both years.

In both years, pH peaked in June and then showed a general tendency of decreasing values towards October at stations 3, 4 and 5, although pH remained over 8. In October, pH values were more similar along the Fleet (Fig. 3.6). At station 7 on 3/10/00 an unusual low pH value of 7.12 was recorded and is likely to be erroneous.

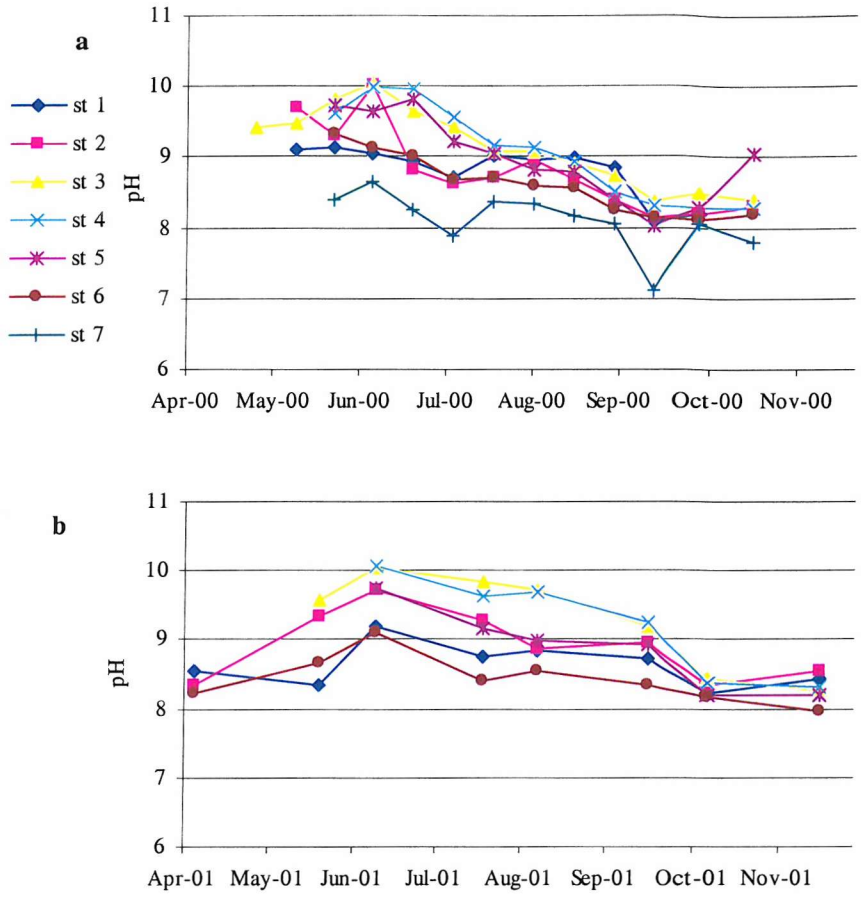


Figure 3.6: pH variation along the Fleet lagoon during 2000 (a) and 2001 (b).

Total alkalinity

During both years there was a clear alkalinity gradient along The Fleet, with higher values observed at station 1 and lower values at stations 6 and 7 (Fig. 3.7). Considering both years, total alkalinity varied between 2224 and 2471 μmol kg^{-1} at stations 6 and 7 and between 2726 and 3553 μmol kg^{-1} at station 1.

Alkalinity versus salinity plots showed that alkalinity was higher at the low salinity end of the Fleet throughout the period studied (Appendices 2 and 3). This behaviour is probably caused by riverine water discharge rich in carbonate and to groundwater seepage from chalk and greensand aquifers underlying the whole area. The apparent non-conservative behaviour showing removal of alkalinity in the mid Fleet during some summer months (e.g. 22/8/00 and 24/7/01) is probably indicative of calcification associated with macroalgae growth. It is unclear why apparent additions of alkalinity were occurring in July 2000 in the mid Fleet but it is possible that calcium dissolution of shells is a contributing factor.

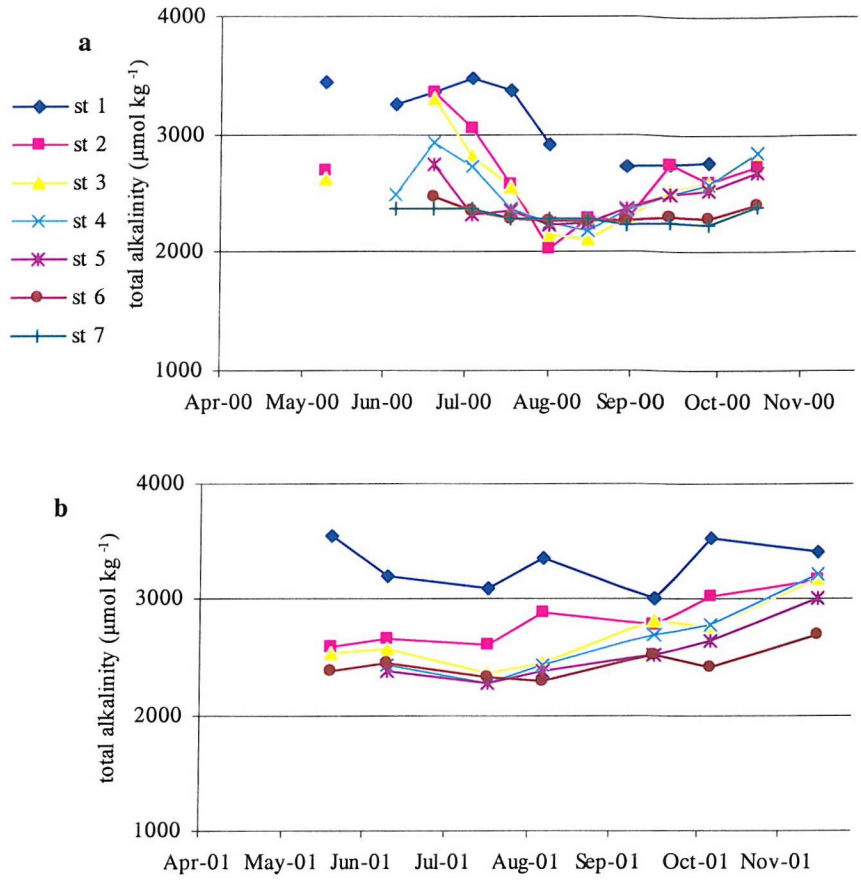


Figure 3.7: Total alkalinity (µmol kg^{-1}) variation along the Fleet lagoon during 2000 (a) and 2001 (b).

Free CO2 (pCO_2)

Free CO_2 represents the concentration of free dissolved CO_2 at equilibrium with the much larger concentration of TCO_2. Station 7 showed higher pCO_2 concentrations during 2000, while the lowest values were recorded at stations 3, 4 and 5 (Fig. 3.8a). From 5/9/00, pCO_2 concentrations increased along the lagoon, peaking on 3/10/00. The extremely high concentration of 161.7 µmol kg^{-1} recorded at station 7 on 3/10/00 (Fig. 3.8a) is likely to be an error related to the low pH value measured at this station on this date (Fig. 3.6a). TCO_2 and pCO_2 were lower in the mid Fleet (stations 3 to 5), suggesting that a process which uses HCO_3^- is taking place in this region of the lagoon. Most macro and microalgae are known to assimilate bicarbonate for photosynthesis, and calcification may also be occurring, but the mechanisms involved in the Fleet are unclear and require further study.

During 2001, higher pCO_2 values were observed at stations 1 and 6, compared to other stations. At station 1, values were higher in 2001, in contrast to the low values

observed at this station during the previous year. Considering the rest of the lagoon, pCO_2 was higher during October (st. 2) and November (st. 3 to 6) (Fig. 3.8b).

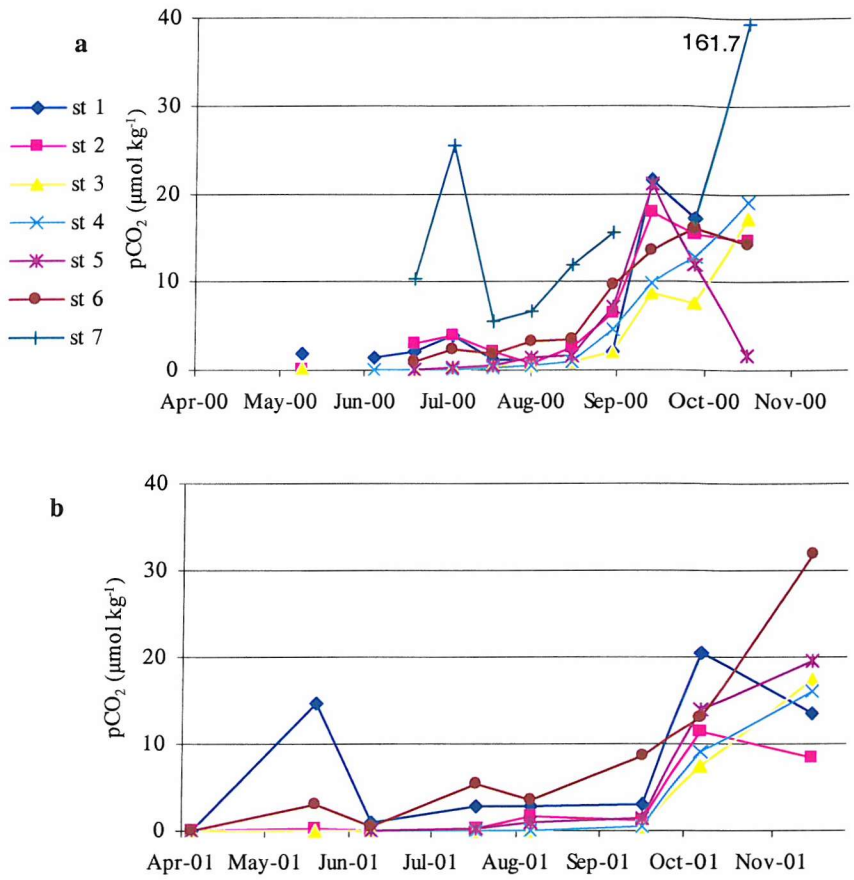


Figure 3.8: pCO_2 (µmol kg^{-1}) variation along the Fleet lagoon during 2000 (a) and 2001 (b).

Total CO_2

Total CO_2 concentrations were lower in the mid Fleet (stations 3 and 4) and increased towards the east and west of the lagoon in both years (Fig. 3.9a, b). Higher values were observed at stations 1, 2, 6 and 7. Riverine input of carbonate increases TCO_2 and alkalinity, and may contribute to the high total CO_2 levels observed at stations 1 and 2. During 2000, total CO_2 values tended to decrease from late July to late August at stations 1, 2 and 7. At stations 3 to 6 total CO_2 remained constant from June to August. Then, from 5/9/00, values increased steadily along the whole lagoon until October, when they were more similar (Fig. 3.9a). This trend is consistent with the rainfall pattern and salinity distribution. Absolute values were quite comparable between both years, but higher values were observed during October and November 2001 at station 1 when compared with the same months the year before (Fig. 3.9b).

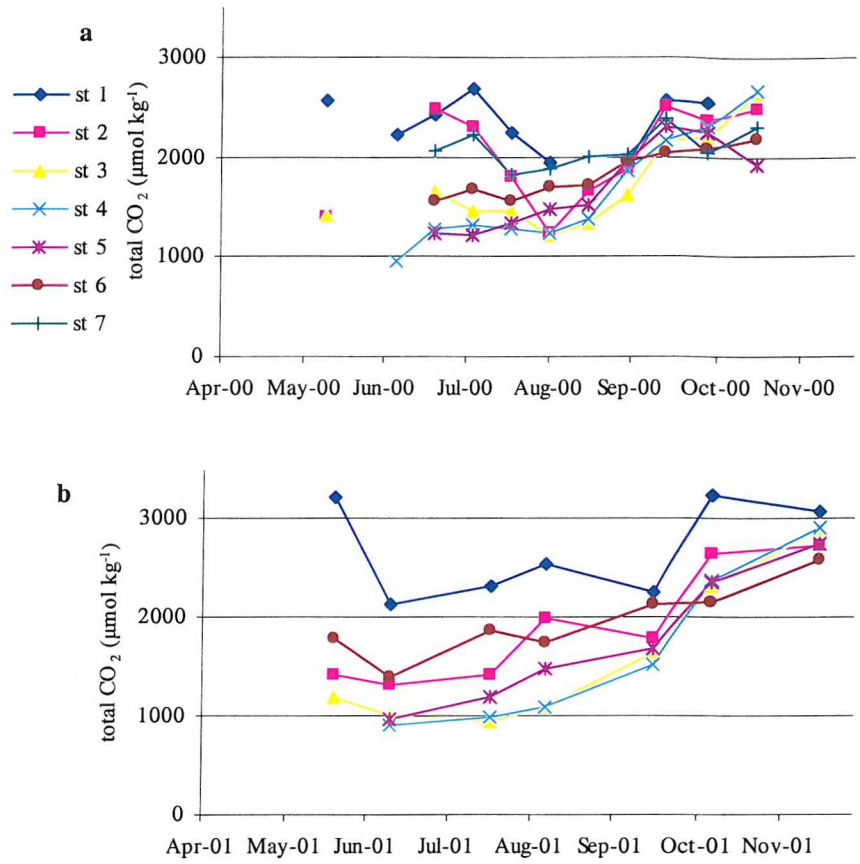


Figure 3.9: Total CO_2 (µmol kg^{-1}) variation along the Fleet lagoon during 2000 (a) and 2001 (b).

3.2.2 Nutrients

Phosphate

There was a clear gradient in phosphate concentrations along the Fleet, with higher concentrations found at stations 1 and 2 and lower ones at stations 6 and 7 (Fig. 3.10). Average concentrations of phosphate for the period studied were 4.2 µM (2000) and 6.2 µM (2001) at station 1 and 0.4 µM (2000) and 0.5 µM (2001) at station 6.

A clear seasonal pattern of variation was observed, particularly at stations 1 and 2, with a peak in phosphate concentrations during the summer in both years. At stations 5 to 7 phosphate concentrations were lower. The sharp decrease observed from 22/8/00 to 5/9/00 was probably caused by phytoplankton assimilation, as chlorophyll-a concentrations increased considerably between those days.

It is noteworthy that higher phosphate concentrations were found in the whole lagoon during 2001 than in 2000 (Fig. 3.10). During 2001, phosphate concentrations built up in the water column from April to August at stations 1 to 4. During summer 2001, phosphate concentrations at station 1 were similar to the previous year, while at station 2 concentrations were considerably higher in 2001. In October and November, higher

phosphate values were observed in 2001, probably as a result of the poor phytoplankton growth during late summer 2001, in comparison to this period in 2000.

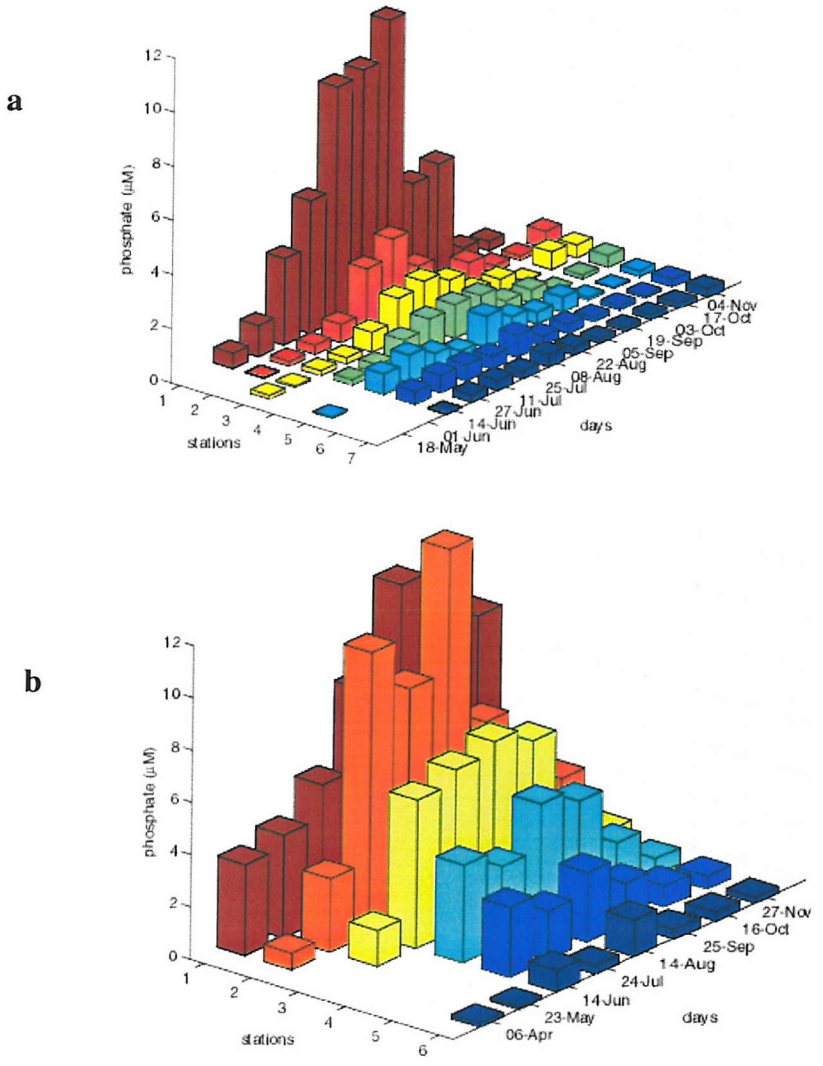


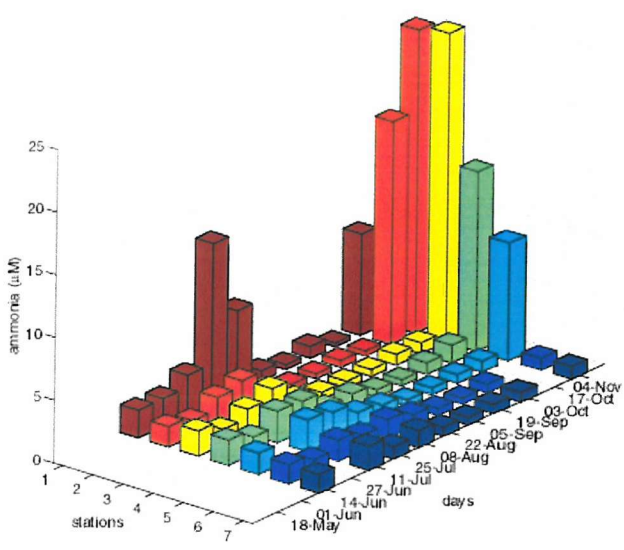
Figure 3.10: Phosphate concentrations in the Fleet lagoon during 2000 (a) and 2001 (b).

Phosphate versus salinity plots for each day of sampling showed that higher phosphate concentrations were associated with the lowest salinity, at station 1 (Appendices 4 and 5). In general during 2000, phosphate concentrations markedly decreased within the salinity range of 27 to 36 (around st. 2), suggesting a marked removal between stations 1 and 2. It is difficult to say why from June to September 2001 there was input of phosphate into station 2, where concentrations were higher than at station 1.

Ammonium

Ammonium concentrations were generally lower than 3 μM in the whole lagoon from April to October. Higher ammonium concentrations were observed at station 1 on 25/7/00 and 8/8/00, when ammonium reached 12.8 μM (Fig. 3.11a). During the same period in 2001, ammonium concentrations did not surpass 5 μM at station 1 (Fig. 3.11b). On 17/10/00, a marked increase in ammonium was observed at stations 1 and 2, up to 17.8 μM, and increased concentrations followed on 4/11/00 at stations 2 to 5, up to 24.6 μM (no data available for station 1). On 27/11/01, ammonium concentrations were not as high as the ones observed on 4/11/00, and it is possible that a peak in ammonium concentrations was missed at the beginning of November in 2001, although concentrations of 11.5 μM were observed at station 1 on 16/10/01.

a



b

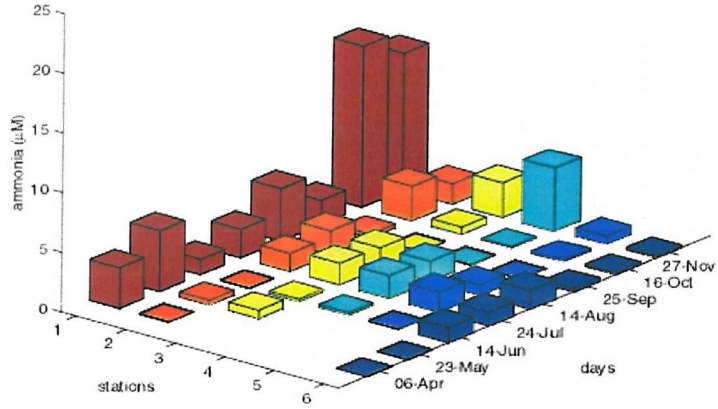


Figure 3.11: Ammonium concentrations in the Fleet lagoon during 2000 (a) and 2001 (b).

Ammonium versus salinity plots showed that ammonium concentrations were quite stable across the salinity gradient of the lagoon and fitted a dilution line on most dates (Appendices 6 and 7). Exceptions to this general pattern were observed on the days of higher ammonium concentrations; 17/10/00 and 4/11/00, when ammonium addition was observed at station 2 and 4. From August to November 2001 a marked removal of ammonium was observed between stations 1 and 2.

Nitrate+Nitrite

Higher nitrate plus nitrite concentrations (from here on refereed to as nitrate) were generally observed at station 1 in both years (Fig. 3.12), particularly during 2001. From June to October, mean nitrate concentration at station 1 was 13.7 μM in 2000 and 47.5 μM in 2001. Comparing the same period, mean values for stations 2 and 3 were much lower, 2.4 μM (st. 2) and 2.5 μM (st. 3) in 2000 in comparison to 2001(9.3 μM at st. 2 and 3.4 μM at st. 3). During 2000, nitrate concentrations at station 1 peaked initially on 27/6/00 (39.0 μM), then decreased to around 10.0 μM during July and August, and were not detectable in September. When the sampling survey was initiated in 2001, 61.8 μM and 52.6 μM of nitrate were recorded at station 1 in April and May respectively, probably due to the increased runoff during these wet months. In contrast, during 2000, station 1 was first visited on 1/6/00, and nitrate had already decreased to 19.6 μM. Comparing nitrate concentrations at station 1 in summer from both years, higher values were measured in 2001, and this may be an effect of the increased river discharge in July and August 2001 relative to the same period in 2000.

At other stations, nitrate was often lower than 1 μM between April and mid October. From mid October to the beginning of November extremely high nitrate values were observed initially at stations 1 to 4 (west Fleet) and then throughout the lagoon in 2000 (Fig. 3.12a). High nitrate concentrations were also observed in mid October 2001 in the west Fleet (Fig. 3.12b). These higher nitrate concentrations were associated with periods of pronounced rainfall.

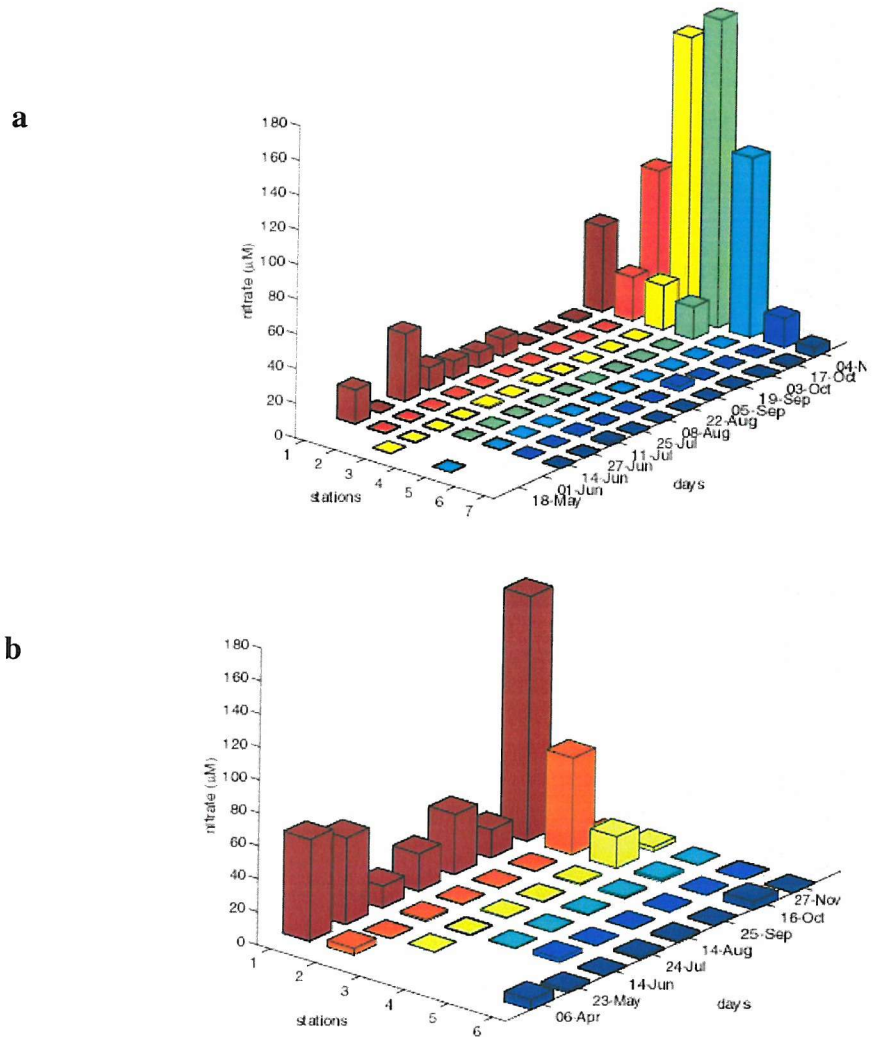


Figure 3.12: Nitrate plus nitrite concentrations in the Fleet lagoon during 2000 (a) and 2001 (b).

Nitrate versus salinity plots for each day of sampling showed that in general higher nitrate concentrations were found at the lower salinity end of the lagoon (Appendices 8 and 9). On most dates nitrate removal was greater than the dilution with seawater. The bloom of cryptophytes in June 2000 and of *P. micans* in September 2000 had a dramatic impact on nitrate concentrations at station 1. By September 2000 nitrate was entering the lagoon from the adjacent coastal water, as shown by increased nitrate concentrations at station 7.

Silicate

During both years, a clear gradient in silicate concentration was observed in the Fleet, with higher concentrations found at station 1, gradually decreasing as salinity

increases towards the mouth of the lagoon (Fig. 3.13). Average silicate concentrations for the period between June and mid October were 69.2 µM and 7.4 µM at stations 1 and 6 respectively in 2000 and 71.2 µM and 4.9 µM in 2001.

A clear seasonal pattern was observed, of lower concentrations during summer, then increased concentrations from August to November, initially only in the west Fleet (station 1) and then gradually towards the mouth of the lagoon. During 2001, increased silicate concentrations were also observed in early April and May at station 1 (Fig. 3.13b), probably due to the extremely wet winter-spring period of 2000/2001. In comparison to 2000, lower silicate concentrations were observed from September to November in 2001 at station 2 to 5 probably due to the lower rainfall during these months in 2001.

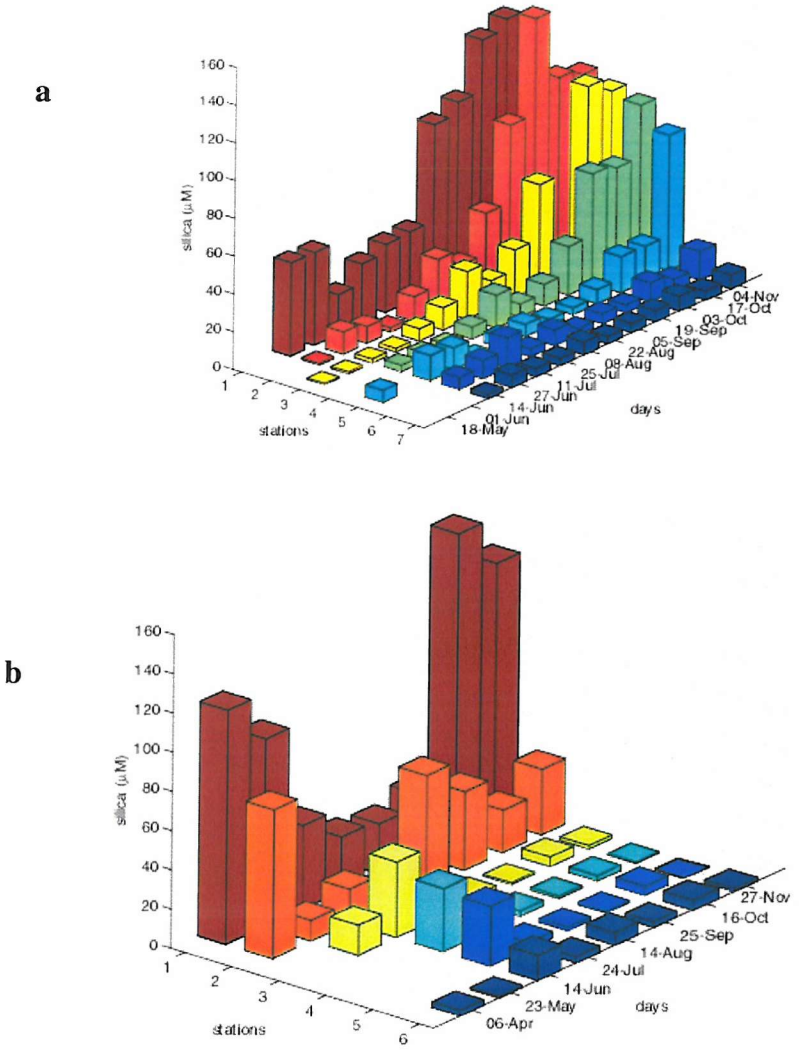


Figure 3.13: Silicate concentrations in the Fleet lagoon during 2000 (a) and 2001 (b).

Salinity versus silicate plots showed that higher silicate values were associated with the less saline end of the lagoon, indicating the more pronounced freshwater input at station 1(Appendices 10 and 11). Silicate concentrations decreased up the salinity gradient, due to removal and dilution with seawater. On 14/6/01 marked silicate removal was observed at station 2.

3.2.3 Light attenuation

During 2000, the light attenuation coefficient (k) values varied between 0.1m^{-1} at station 4 on 5/9/00 and 6.4 m^{-1} at station 2 on 3/10/00 (Table 3.1). The mean k value during 2000 was 1.9 m^{-1}.

During 2001, k values varied between 0.5 m^{-1} at station 6 on 16/10/01 and 19.0 m^{-1} at station 2 on 27/11/01. Mean k value during 2001 was 3.0 m^{-1} and the highest values observed on 27/11/01 were due to resuspension of bottom sediments caused by wind mixing at stations 1 to 4 and increased chlorophyll-a concentrations. It was necessary to filter these samples through a 200 and a 40 μm mesh to be able to count phytoplankton cells in 10 mL sedimented samples. In contrast, during 2000, higher k values (3.7-6.4 m^{-1}) were observed in September and October and were associated with high chlorophyll-a concentration. The euphotic zone depth markedly exceeded the mean depth of the lagoon on most dates from both years and incident light was not a limiting factor due to the extreme shallowness of the lagoon. On average, 2.7% of the surface light reached the depth of 1 m during 2001.

Table 3.1: Light attenuation coefficient (k) measured in the Fleet lagoon during 2000 and 2001 (units m^{-1}).

Station	27/6/00	11/7/00	25/7/00	8/8/00	22/8/00	5/9/00	19/9/00	3/10/00	17/10/00	4/11/00
1	1.15			1.75	1.20		4.79	5.29	0.80	
2			1.35	1.43	2.07	0.23	2.11	6.44	1.40	0.81
3		2.59	2.02	4.51	2.51		1.30	2.24	1.65	2.93
4		4.34		1.01	1.91	0.09	1.24	2.36	2.45	3.01
5			0.16	3.70	0.53	0.16	0.89	4.16	2.23	5.42
6	0.50	0.80	0.27	1.16	1.27	0.81	0.39	0.82	0.88	3.54

Station	23/5/01	14/6/01	24/7/01	14/8/01	25/9/01	16/10/01	27/11/01			
1	5.7		1.8	1.1	1.1	2.7	12.6			
2	2.2	2.3	1.5	0.9	1.6	1.1	19.0			
3	1.2	2.9	1.6	1.6	2.9	0.7	16.2			
4			1.3	1.4	1.3	1.7	9.2			
5			1.0	1.0	0.6	0.8	4.0			
6	1.8	2.5	0.9	1.3	0.6	0.5	1.2			

A significant ($p < 0.01$) correlation was found between chlorophyll-a concentrations and k values during 2000 ($r^2 = 0.414$) and 2001 ($r^2 = 0.699$). The specific vertical attenuation coefficient per unit phytoplankton concentration is known to vary widely, according to cell size and geometry (package effect), pigment content and profile and the ratio of other photosynthetic pigments to chlorophyll-a (Kirk, 1983). The colour of the aquatic medium in which the cells are suspended can also have a marked influence on the values of the vertical attenuation coefficient per unit phytoplankton concentration, as well as variations in the light-scattering properties of different types of phytoplankton cells (Kirk, 1983). Seaweed and eelgrass biomass and sediment resuspension will also have an effect on light scatter and penetration in the waters of the shallow Fleet lagoon.

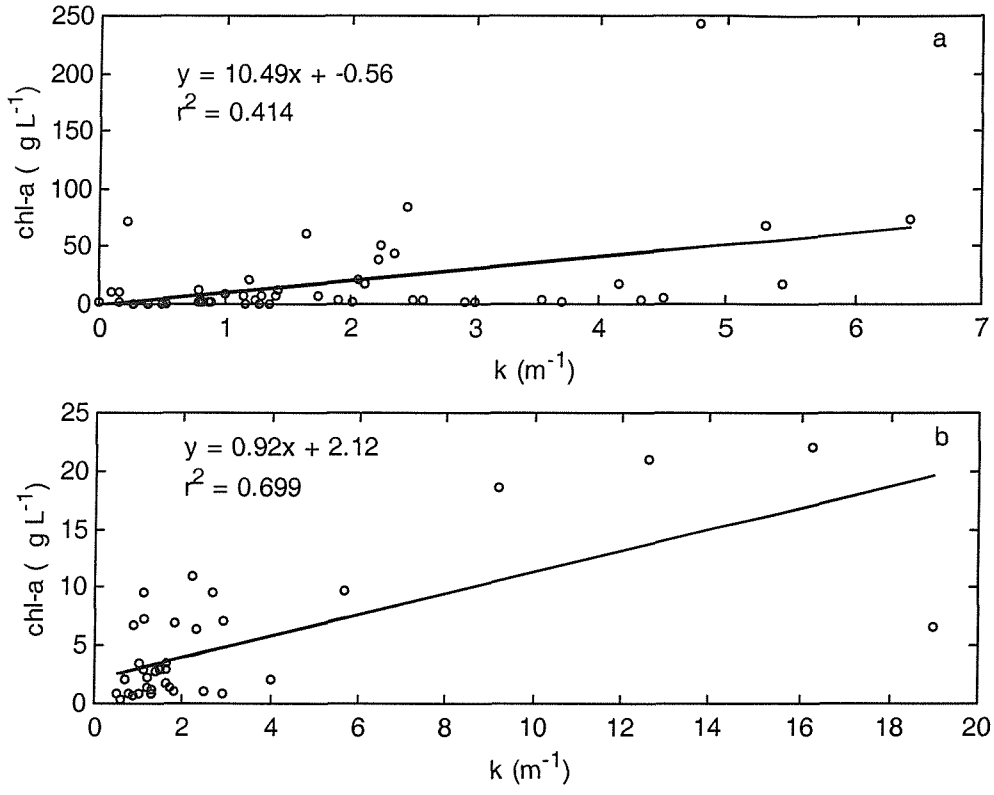


Figure 3.14: Chlorophyll-a versus the attenuation coefficient (k) at the Fleet lagoon on 2000 (a) and 2001 (b).

3.2.4 Chlorophyll-a

During both years there was a clear gradient of chlorophyll-a concentrations along the Fleet, with higher values recorded at station 1 (Fig. 3.15). Mean values for stations 1 and 6 were respectively 60 μg L^{-1} and 1.1 μg L^{-1} in 2000 and 48.6 μg L^{-1} and 1.2 μg L^{-1} in 2001.

During 2000, two major peaks of chlorophyll-a were observed at station 1. The first one on 1/6/00 (163 μg L^{-1}) was caused by high abundances of cryptophytes and the

second one from 22/8/00 to 3/10/00 (peak of 244 μg L^{-1}), caused by an exceptional bloom of the dinoflagellate *Prorocentrum micans*, that visibly discoloured the waters of the lagoon. High chlorophyll-a values of 72 and 74 μg L^{-1} were also observed at station 2 on 5/9/00 and 3/10/00 respectively. Lower chlorophyll-a values were measured between these dates, on 19/9/00 (17 μg L^{-1}), and this decreased value might have been caused by tidal (spring tides) and wind advection of the *P. micans* bloom towards the Abbotsbury embayment, as chlorophyll-a was extremely high at station 1 on this date. Lower chlorophyll-a concentrations were observed at station 1 from June to early August (Fig. 3.15a). Nitrate and phosphate concentrations were not likely to be limiting, thus it is unclear why conditions prevented phytoplankton growth during these months.

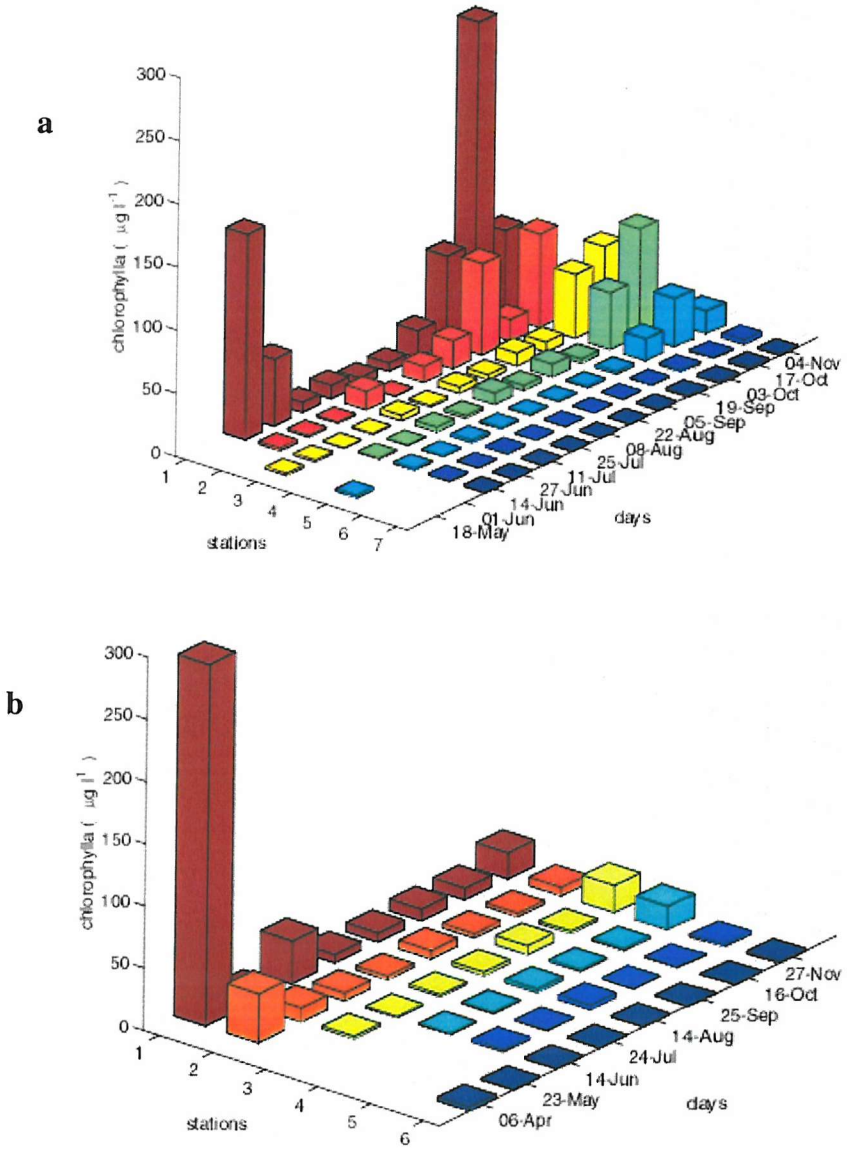


Figure 3.15: Chlorophyll-a concentration in the Fleet lagoon during 2000 (a) and 2001 (b).

At stations 3 to 5 chlorophyll-a values were lower than 5.2 μg L^{-1} from May to September 2000, while at stations 6 and 7 chlorophyll-a did not change markedly, and only exceeded 2 μg L^{-1} on 4/11/00, when 3.5 μg L^{-1} was recorded at station 6. Greater chlorophyll-a concentrations were measured at stations 3 and 4 from 5/9/00 and peak values at these stations were observed during October. At station 5, increased chlorophyll-a was observed from 3/10/00 to 4/11/00. The increased chlorophyll-a values from west to east of the lagoon during September to November 2000 represent the advective flushing of the *P. micans* bloom towards the mouth of the lagoon during this period caused by increased freshwater inputs at the head of the lagoon (Fig. 3.15a).

During 2001, a chlorophyll-a peak of 290.4 μg L^{-1} was observed early in the year, on 6/4/01, representing extremely high abundances of cryptophytes. Comparable chlorophyll-a values were observed during both summers studied, however, much lower chlorophyll-a concentrations were found at stations 1 and 2 from August to October 2001, relative to 2000. On 27/11/01, chlorophyll-a concentrations increased to about 20 μg L^{-1} at stations 1, 3 and 4 (Fig. 3.15b), and this was likely to be caused by resuspension of microphytobenthos, as there were strong winds on this day.

3.2.5 Particulate organic carbon and nitrogen

Particulate organic carbon (POC) and nitrogen (PON) were only measured during the 2000 survey. As for some of the other parameters, relatively higher concentrations of POC and PON were detected at station 1. POC and PON include live material, as phytoplankton and bacteria, and a detrital fraction, e.g. cell debris in suspension. Mean POC at station 1 was 8908 μg L^{-1}, and values at this station varied between 1713 μg L^{-1} on 8/8/00 and 48341μg L^{-1} on 19/9/00, during the peak of chlorophyll-a at this station, suggesting that phytoplankton represented an important component of the POC. In contrast, mean POC at station 7 was 493 μg L^{-1}, and ranged between 200 and 914 μg L^{-1}. At the other stations, intermediary concentrations were observed and POC gradually decreased from station 1 to 7 (Fig. 3.16). POC showed a significant correlation with chlorophyll-a ($POC = 150.5(chl\text{-}a) + 583.2$, $r^2=0.895$, $p < 0.01$) (Fig. 3.17).

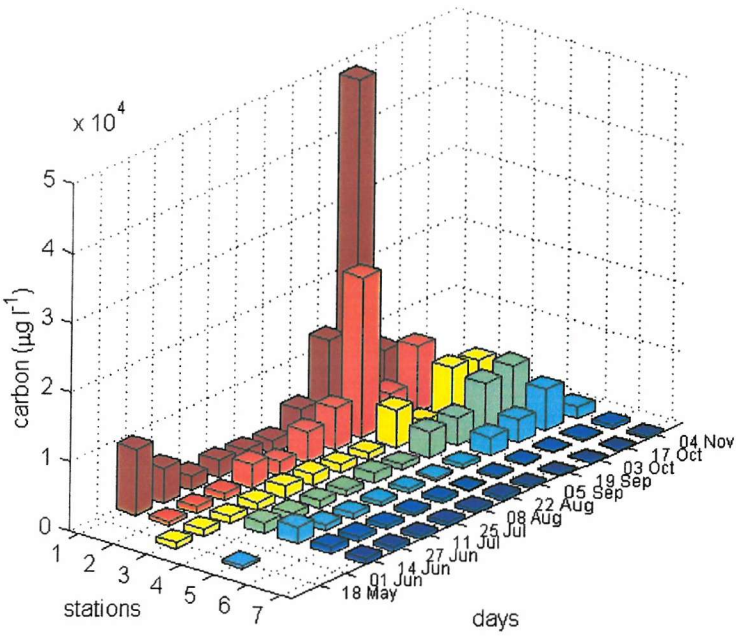


Figure 3.16: Particulate organic carbon (POC) concentrations in the Fleet lagoon during 2000.

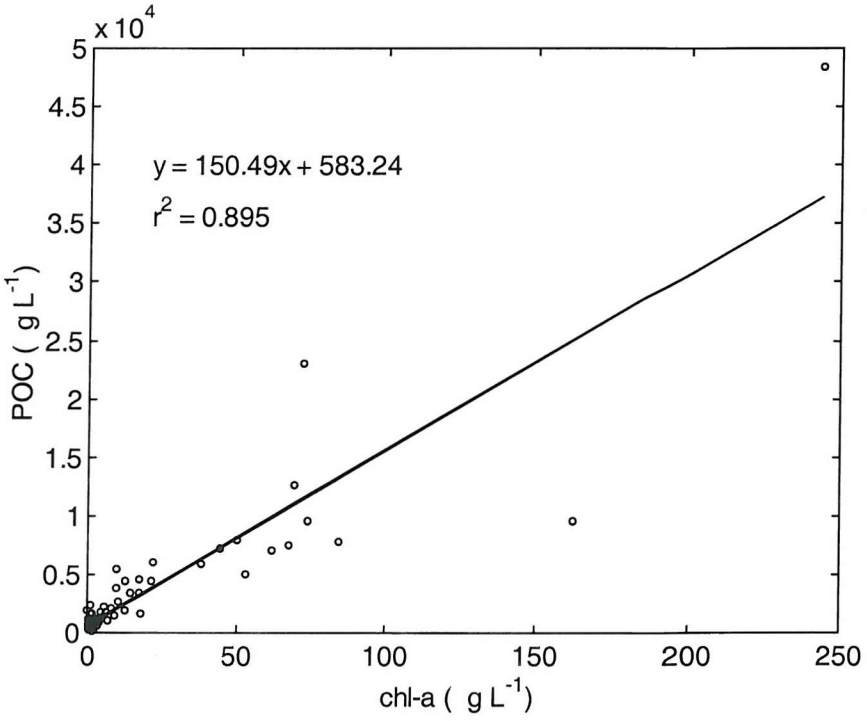


Figure 3.17: POC (µg L^{-1}) versus chlorophyll-a (µg L^{-1}) at the Fleet lagoon during 2000.

Mean PON at station 1 was 1122 µg L^{-1} and varied from 242 µg L^{-1} on 27/6/00 to 4724 µg L^{-1} on 19/9/00. At the other end of the lagoon, at station 7, PON varied between 22 µg L^{-1} and 130 µg L^{-1}. Mean concentration at this station was 57 µg L^{-1} and a gradual

decrease in PON was observed from station 1 to 7 (Fig. 3.18). PON temporal variation followed that of POC with both parameters peaking at the same time as chlorophyll-a at station 1, on 1/6/00 and 19/9/00. Mean particulate C:N ratio for the whole lagoon ranged between 5 and 19 and the mean was 9 (Fig. 3.19).

This value is close to the Redfield ratio, of 7.2 for particulate matter in the sea, especially if considering that a coastal lagoon will receive a high input of land material, that show a distinct stoichiometry from particulate matter in the ocean.

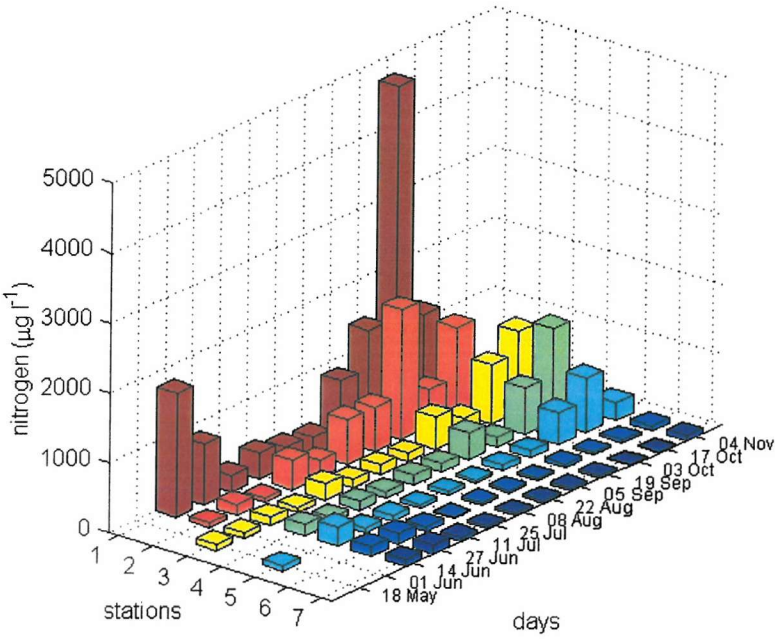


Figure 3.18: Particulate organic nitrogen concentrations in the Fleet lagoon during 2000.

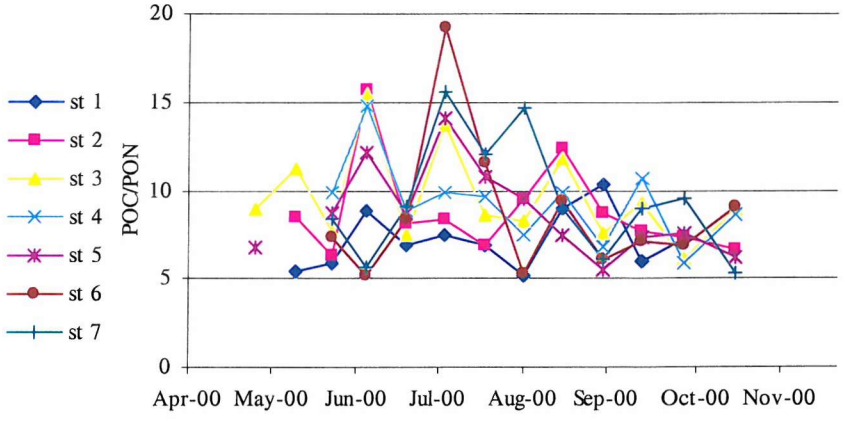


Figure 3.19: POC to PON mass ratio variation along the Fleet lagoon during 2000.

3.2.6 Phytoplankton abundance

Phytoplankton abundance showed a similar pattern to chlorophyll-a concentrations. A gradient of increasing total abundance and biomass was observed from station 7 to station 1. The sampling stations along the lagoon showed differences in the temporal pattern of species dominance and composition.

Year 2000

During 2000, small sized phytoflagellates were the most abundant group throughout the whole lagoon. They included two species of cryptophytes, tentatively identified (as it has been always difficult to identify different groupings within the Cryptophyceae, and the use of electron microscopy is usually necessary, Hill, 1991) as *Hilea* cf. *fusiformes*, here called *Cryptomonas* sp. and *Teleaulax* cf. *acuta*, here called *Cryptomonas* sp.2, *Eutreptiella marina* and small flagellates 6 μm long. Dinoflagellates at the lower end of size range of the microphytoplankton (up to 40 μm) followed as a dominant group from stations 1 to 6, while station 7 was the only one where diatoms were more abundant than dinoflagellates (see Table 3.7). In general, phytoflagellate (particularly *Cryptomonas* sp. and *E. marina*) and dinoflagellate dominance were separated temporally, and the first group dominated from May to mid July, while the latter one from mid July to October at stations 1 to 6.

The abundance of species of microzooplankton, i.e. the ciliates *Strombidium* spp. and the heterotrophic dinoflagellates *Crypthecodinium* cf. *cohnii* and *Oxyrrhis marina* are also included in this section. *Crypthecodinium cohnii* has been reported to have 52 sibling species, 7 major and 45 that were only found once (Steindinger and Tangen, 1997).

At station 1, the abundance of *Cryptomonas* sp., *E. marina* and the heterotrophic dinoflagellate *Crypthecodinium* cf. *cohnii* showed the same temporal pattern, peaking on 1/6/00 and then markedly decreasing after this day (Fig. 3.20a). *Cryptomonas* sp. was the dominant species on this date (52170 cells mL^{-1}, 163 μg chl-a L^{-1}). It is possible that the peak in cell abundance of this species occurred earlier in the year, and that by the 1/6/00 it was already declining, but the sampling program was started at this station only on this date. From late July to late August, lower total cell abundances were observed, and a short lived bloom of the dinoflagellate *Scrippsiella* sp. was observed in late August, when cell abundances reached 263 cells mL^{-1}. During September, an exceptional bloom of the dinoflagellate *Prorocentrum micans* reached cell abundances up to 43623 cells mL^{-1} on 19/9/00 (chl-a of 243.8 μg L^{-1}). This bloom seems to have been concentrated towards station 1 on this date by wind (see Fig. 3.28a and 3.29a) and tidal advection.

At the same time as the *P. micans* bloom, an unidentified athecate dinoflagellate 20 μm long was also abundant, reaching 1866 cells mL^{-1}. Chains of two cells of this athecate dinoflagellate were commonly observed in these samples. This behaviour and the dimensions of this species may suggest that this dinoflagellate is *Gymnodinium nolleri*, whose cysts are morphologically very similar to cysts of *Gymnodinium catenatum*, and were reported to be common in Denmark (Ellegaard et al. 1998). Although cultures of this species were established from isolated cysts from Denmark, the motile cells were never reported in Scandinavia (Ellegaard et al. 1998). However, classification of this species is only tentative, as important morphological features of unarmored (naked or athecate) dinoflagellates are often obliterated upon preservation and they are considered "non-preservable" species (Larsen and Sournia, 1991). A small diatom, *Minidiscus* sp. was also abundant (1400 cells mL^{-1}) at station 1 on 19/9/00. Much lower total cell abundances were observed during October at this station and small flagellates (6 μm long) became more abundant.

At station 2, *Cryptomonas* sp. was the dominant species in June, but peak cell abundances were lower relative to the ones observed at station 1 (1023 cells mL^{-1}). On 27/6/00 the diatoms *Licmophora* sp., *Cylindrotheca closterium* and other pennate diatoms not identified to genus level were the most abundant species. However, on the next sampling date, on 11/7/00, high densities of *E. marina* were observed (4456 cells mL^{-1}), together with a mixed assemblage of dinoflagellates (Fig. 3.20b). Among the dinoflagellates, three athecate forms approximately 11, 20 (possibly *G.* cf. *nolleri*) and 30 μm long were the most abundant, and showed cell abundances of 85, 898 and 212 cells mL^{-1} respectively. The heterotrophic *C.* cf. *cohnii* (263 cells mL^{-1}), *Scrippsiella* sp. (76 cells mL^{-1}), *P. micans* (68 cells mL^{-1}) and *Gyrodinium instriatum* (42 cells mL^{-1}) were also present on this day. From 11/7/00, *P. micans* showed increasing cell numbers at station 2 and during August and September exceptionally high abundances of this dinoflagellate were observed, peaking on 5/9/00 (25661 cells mL^{-1}). *Scrippsiella* sp. was also abundant during this period, reaching densities of 941 cells mL^{-1}. By 3/10/00, *P. micans* was still the dominant species, but on 4/11/00 much lower phytoplankton abundance was observed at station 2 (29 cells mL^{-1}).

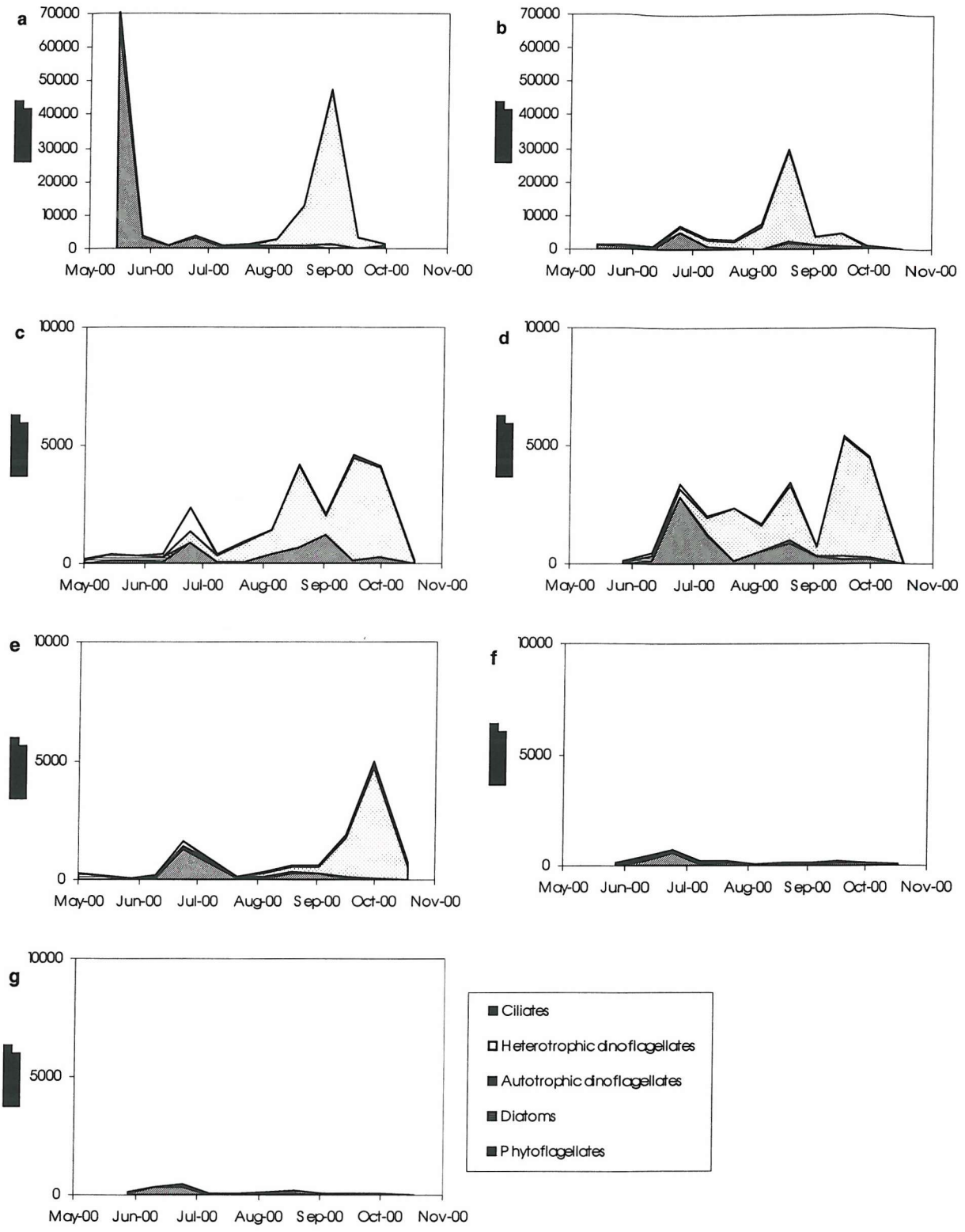


Figure 3.20: Major phytoplankton groups total abundances at stations 1 to 7 (a to g) in the Fleet lagoon during 2000.

Lower cell numbers were observed at station 3, relative to the previous two stations (Fig. 3.20c). Sampling was started earlier in the year at this station and from 18/5/00 to 27/6/00, low phytoplankton abundance was observed, and total cell numbers varied between 194 and 411 cells mL^{-1}. Pennate diatoms were the dominant group during

this period, and *Cryptomonas* sp. followed in abundance. On 11/7/00, total phytoplankton abundance increased to 2395 cells mL^{-1} and was characterized by a mixed assemblage of dinoflagellate species, including the heterotrophic species *Oxyrrhis marina* (747 cells mL^{-1}) and *C.* cf. *cohnii* (237 cells mL^{-1}), *P. micans* (195 cells mL^{-1}), a non identified thecate (127 cells mL^{-1}), *Scrippsiella* sp. (51 cells mL^{-1}), the athecate species of 11 and 20 μm (68 and 25 cells mL^{-1}) and *G. instriatum* (15 cells mL^{-1}). *E. marina* (674 cells mL^{-1}) was also abundant on this date and *Cryptomonas* sp. was found at this station during all sampling dates except for 18/5/00, showing abundances that varied between 26 (4/11/00) and 1064 cells mL^{-1} (19/9/00). From 11/7/00 to 22/8/00 a mixed dinoflagellate assemblage was observed and later on *P. micans* became the dominant species. On 19/9/00 a decrease in *P. micans* abundance was observed, and this may have been caused by the concentration of this dinoflagellate towards station 1. *P. micans* abundance at station 3 was highest on 3/10/00 (4257 cells mL^{-1}) and was still high on 17/10/00 (3737 cells mL^{-1}). During November, lower total cell numbers were observed (156 cells mL^{-1}), and *P. micans* shared dominance with the autotrophic ciliate *Myrionecta rubra.*

Sampling at station 4 was initiated on 14/6/00. On this date low numbers of cells were observed and total phytoplankton abundance was 118 cells mL^{-1}. By late June, increased abundances were observed and the dominant species were *Cryptomonas* sp., an un-identified pennate diatom and the heterotrophic dinoflagellate *O. marina* (Fig. 3.20d). As observed at station 3, *Cryptomonas* sp. was a constant component of the plankton at station 4, except for 8/8/00 and 4/11/00, and was the dominant species during July (peak of 1209 cells mL^{-1}). From 11/7/00 onwards dinoflagellates became increasingly more abundant and a mixed assemblage similar to that observed at station 3 was present. The 20 μm unidentified athecate dinoflagellate was the dominant species on 8/8/00 (1119 cells mL^{-1}), followed by *P. micans* (712 cell.mL^{-1}), *Scrippsiella* sp. (237 cells mL^{-1}), the athecate species of 11 and 30 μm (51 and 34 cells mL^{-1}) and an un-identified thecate dinoflagellate (34 cells mL^{-1}). As observed at station 3, from 22/8/00 *P. micans* became the dominant species. On 19/9/00 a decrease in *P. micans* abundance was observed also at this station, however, during October, *P. micans* peaked at station 4 (5021 cells mL^{-1}). During November, lower total cell abundances were found (55 cells mL^{-1}), and *P. micans* shared the dominance with the ciliate *M. rubra*, as observed at station 3.

At station 5, pennate diatoms, not identified to the genus level, were dominant on 18/5/00, representing 93% of the total abundance of 274 cells mL^{-1}. During June, low phytoplankton abundance occurred at this station, however, on 11/7/00, *Cryptomonas* sp. (1011 cells mL^{-1}) and *E. marina* (266 cells mL^{-1}) became more abundant (Fig 3.20e).

Pennate diatoms and the mixed dinoflagellate assemblage also developed, however abundances were low relative to stations 3 and 4. During August, a small athecate dinoflagellate (11 μm) was the dominant species (peak of 137 cells mL^{-1}), followed by *Cryptomonas* sp. (53 cells mL^{-1}) and *P. micans* (29 cells mL^{-1}). *P. micans* dominance at this station was observed later in the year, only on 19/9/00 and peak values of 4623 cells mL^{-1} were found on 17/10/00. On the last day of sampling (4/11/00), cell abundance was relatively high (709 cells mL^{-1}), and dominated by *P. micans* followed by *M. rubra*, as observed at stations 3 and 4. Small flagellates were always present from July to October at station 5.

At station 6, total phytoplankton abundance was low when compared with other stations, and varied between 94 cells mL^{-1} on 22/8/00 and 694 cells mL^{-1} on 11/7/00 (Fig 3.20f). On 14/6/00, an unidentified thecate dinoflagellate measuring 35 μm was the dominant species (74 cells mL^{-1}), followed by *M. rubra* (43 cells mL^{-1}). On 27/6/00 and 11/7/00 *Cryptomonas* sp. was the dominant species (368 cells mL^{-1}), and on the later date together with *E. marina* (198 cells mL^{-1}). The small athecate dinoflagellate (11 μm) was the more abundant dinoflagellate at this station from 27/6/00 until 19/9/00 and very low abundances of *C.* cf. *cohnii*, *Scrippsiella* sp., *P. micans* and a non identified 20 μm thecate were also observed. An athecate dinoflagellate measuring approximately 21μm long and resembling *Karenia mikimotoi* was observed at this station only on 3/10/00 at cell abundances of 20 cells mL^{-1}. *P. micans* became the dominant species at this station on 4/11/00, when the bloom finally reached station 6. Several diatom species were observed at this station, *Nitzschia* sp., *Guinardia striata*, *C. closterium* and *Licmophora* sp., although cell numbers were low compared to other stations. Small flagellates were present from July to October.

At station 7 total phytoplankton abundance was comparable to station 6 (Fig. 3.20g). Pennate diatoms were more numerous on 14/6/00 and were succeeded by *Cryptomonas* sp. and *E. marina* on 27/6/00 and 11/7/00. On 25/7/00 and 8/8/00 low cell abundances were observed and *Cryptomonas* sp., probably originating from inside the lagoon, was the dominant species. On 5/9/00, *Licmophora* sp. and other pennate diatoms became more abundant. On 3/10/00, the athecate dinoflagellate resembling *K. mikimotoi* was observed at this station, suggesting that it was coming from Portland Harbour, and not growing inside the lagoon, as this species was only observed at stations 6 and 7 on the same date. *P. micans* was observed at this station on 4/11/00, when the bloom finally reached the mouth of the lagoon.

Year 2001

During 2001 the relative contribution of dinoflagellates to total phytoplankton abundance diminished, and ciliates became more important as a group along the whole lagoon (Fig. 3.21). As samples were collected only monthly during 2001, inferences about species composition during the period between each sampling date will not be made, as phytoplankton reproduce in time scales ranging between hours to days and in the space of a month many changes in the community composition will have occurred and will then be missed in a monthly survey.

On 6/4/01, a monospecific bloom of *Cryptomonas* sp. was observed at station 1, reaching exceptionally high densities of 425734 cells mL^{-1}(Fig. 3.21a). On 23/5/01 a much lower total abundance was observed, and *Cryptomonas* sp. (236 cells mL^{-1}), *M. rubra* (206 cells mL^{-1}) and the heterotrophic dinoflagellate *C.* cf. *cohnii* (100 cells mL^{-1}) were the main species present. High densities of *C.* cf. *cohnii* were observed on 14/6/01 (3773 cells mL^{-1}), 24/7/01 (2009 cells mL^{-1}) and 14/8/01 (1356 cells mL^{-1}), when *Cryptomonas* sp. (6364 cells mL^{-1}) and pennate diatoms (1178 cells mL^{-1}) became more abundant. The high *P. micans* abundance observed during this time in the previous year did not occur in 2001, when *P. micans* was not recorded at station 1. On 3/9/01 samples were collected only from station 1, when a YSI multiprobe was deployed at this site. On this date, high abundances (10288 cells mL^{-1}) of the small diatom *Minidiscus* sp. were recorded, as well as *Cryptomonas* sp. (2814 cells mL^{-1}). *Scrippsiella* sp. was observed at around 200 cells mL^{-1} on 14/6/01 and 3/9/01. On 25/9/01 *Cryptomonas* sp. was the dominant species (4344 cells mL^{-1}), followed by *E. marina* (1428 cells mL^{-1}) and *Minidiscus* sp. (695 cells mL^{-1}). *C.* cf. *cohnii* was abundant on 16/10/01 (700 cells mL^{-1}) and on 27/11/01 *Cryptomonas* sp. became the dominant species again followed by *E. marina.*

At station 2, *Cryptomonas* sp. was the dominant species during the whole year, apart from 14/8/01, when it was replaced by *M. rubra* (Fig. 3.21b) Bloom abundances of *Cryptomonas* sp. were observed on 6/4/01 (27288 cells mL^{-1}), 23/5/01 (51758 cells mL^{-1}), 14/6/01 (59048 cells mL^{-1}) and 24/7/01 (7779 cells mL^{-1}). *E. marina* was observed on 25/9/01 and 27/11/01. Among the dinoflagellates, *Scrippsiella* sp. was recorded on 24/7/01 (247 cells mL^{-1}), the heterotrophic *C.* cf. *cohnii* was present several times between April and November, as well as a small (11 μm) athecate species and the heterotrophic *O. marina*, but always at low abundances.

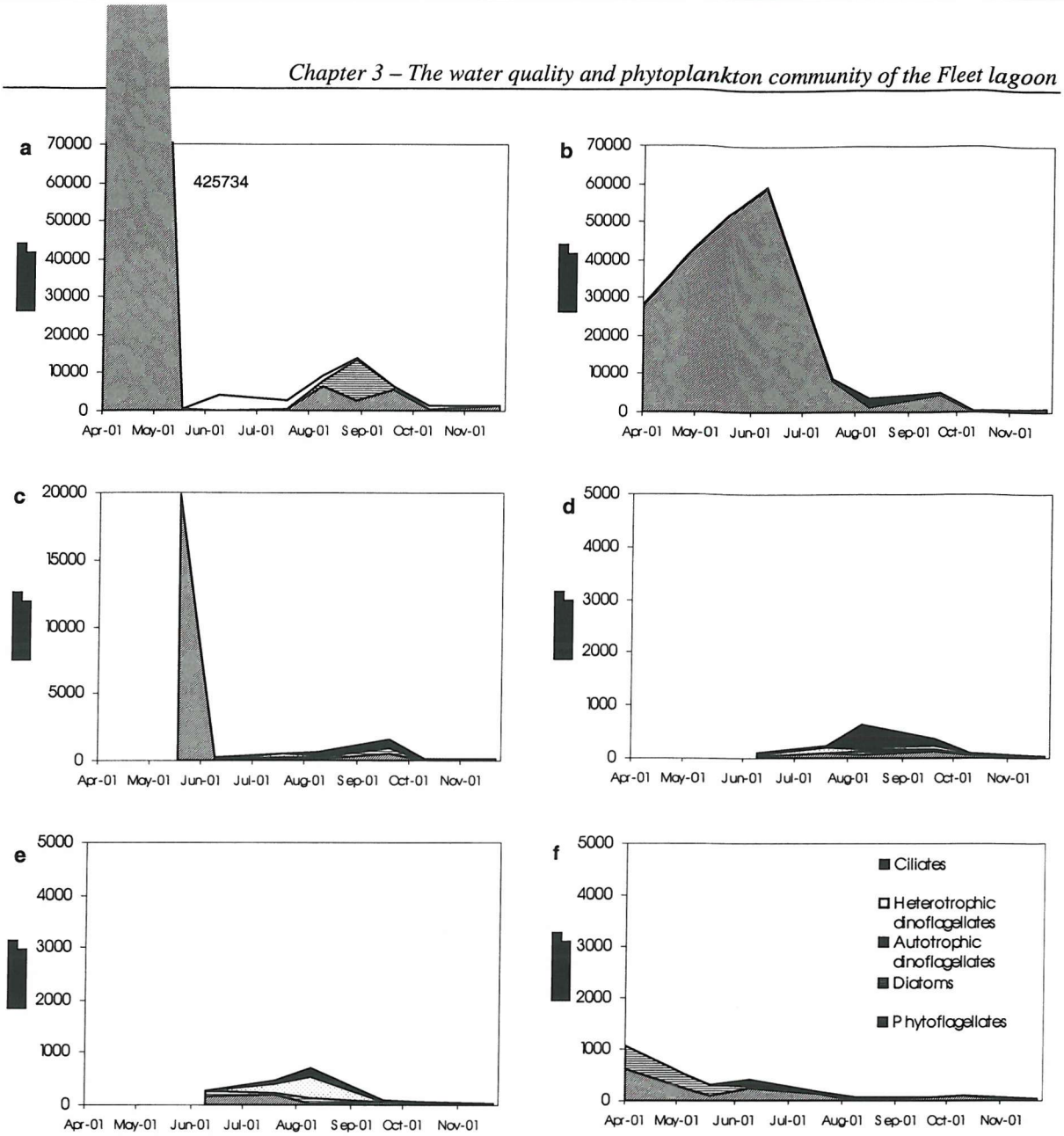


Figure 3.21: Major phytoplankton groups total abundance at stations 1 to 6 (a to f) in the Fleet lagoon during 2001.

Sampling at station 3 was initiated on 23/5/01, and on this date *Cryptomonas* sp. was the dominant species (18662 cells mL^{-1}) (Fig. 3.21c). In contrast to station 2, at station 3 on 14/6/01 much lower phytoplankton abundances were observed (211 cells mL^{-1} in total), representing small flagellates (6 μm), *Scrippsiella* sp., *M. rubra* and *Strombidium* sp.1. On 24/7/01 and 14/8/01, pennate diatoms were observed, as well as a small (11 μm) athecate dinoflagellate and small flagellates. On the later date, *M. rubra* and *G. instriatum* were also abundant. On 25/9/01, *M. rubra* became the dominant species (557 cells mL^{-1}), followed by *Cryptomonas* sp., small flagellates and lower abundances of *Nitzschia* sp., *Licmophora* sp., *O. marina* and the small (11 μm) athecate dinoflagellate. On 16/10/01 and 27/11/01 low phytoplankton abundances were recorded and were represented by

Cryptomonas sp., *E. marina*, *O. marina* and pennate diatoms on the first date and mainly by *M. rubra* on the later one.

Low phytoplankton abundance was observed at stations 4 and 5 during 2001, relative to 2000. Maximum total phytoplankton abundance at stations 4 and 5 during 2001 were respectively 623 and 715 cells mL^{-1}, compared to 5394 and 4958 cells mL^{-1} during 2000. At both stations, *Cryptomonas* sp. and small flagellates (6 μm) were important components of the phytoplankton and the small (11 μm) athecate dinoflagellate was abundant on 24/7/01 and 14/8/01 in both stations (Fig. 3.21d, e). Ciliates (*M. rubra*, *Strombidium* sp. 1 and sp. 3) were the dominant group at station 4 in August, September and November, while *Cryptomonas* sp. and *E. marina* were the dominant species in October. Low abundances of *Cryptomonas* sp. dominated the phytoplankton community of station 5 during September and October, while *Strombidium* sp. 1 was the main species in November (Fig. 3.21e).

At station 6, *Cryptomonas* sp. and *C. closterium* were the dominant species on 6/4/01 (Fig. 3.21f). On 23/5/01 high *Chaetoceros* sp. abundance was found at this station. It is likely that this species entered the lagoon from Portland Harbour, as it was recorded only at this station. High abundances of small flagellates (6 μm) were observed on 14/6/01. *Cryptomonas* sp. followed in dominance on 24/7/01 and then *M. rubra* on 14/8/01. During September and October, *Cryptomonas* sp. was the main species and in November, diatoms were the dominant group, although total abundance was as low as 37 cells mL^{-1}.

P. micans was the most abundant species from August to November 2000, however, this dinoflagellate did not bloom during 2001. In contrast, *P. micans* was not recorded at stations 1 and 2 during 2001 and in the absence of this species, the heterotrophic *C.* cf. *cohnii*, that was abundant at station 1 from 14/6/01 (3773 cells mL^{-1}), maintained high abundances through 24/7/01 (2009 cells mL^{-1}) and 14/8/01 (1356 cells mL^{-1}) at this station, becoming less abundant on 3/9/01 (220 cells mL^{-1}) when the diatom *Minidiscus* sp. was the dominant species (10288 cells mL^{-1}). At station 2, *M. rubra* was the dominant species in August 2001, while during this same month in the previous year, *P. micans* was starting to bloom. The highest abundance attained by *P. micans* during 2001 was observed at station 5 (16 cells mL^{-1} on 24/7/01 and 23 cells mL^{-1} on 14/8/01), and was three orders of magnitude lower than the maximum observed at stations 1 and 2 in the previous year. The possible reasons behind the interannual variation in development of *P. micans* blooms in the Fleet lagoon will be discussed in a later section.

3.2.7 Phytoplankton carbon content

The phytoplankton carbon content was calculated based on linear dimensions of the main species present in the lagoon. Calculations of biovolume and carbon content were made using published equations (see section 2.1.5). Table 3.2 lists the length and width measurement, cell biovolume and carbon content of species from the Fleet lagoon.

Table 3.2: Linear dimensions, cell biovolume (μm^3) and carbon content of main phytoplankton and microzooplankton species from the Fleet lagoon.

Species	Length	Width	Biovolume	pg C $cell^{-1}$
Autotrophic dinoflagellates				
Prorocentrum micans	30.2	18.8	11708	1428
Prorocentrum lima	38.4	28.4	16209	1938
Scrippsiella sp.	29.2	26.2	10474	1286
Athecate 11 μm	9.3	11.5	522	77
Athecate 20 μm	22.2	20.8	5380	688
Athecate 30 μm	30.3	30.6	14690	1767
Gyrodinium instriatum	33.3	26.5	12211	1486
Karenia cf *mikimotoi*	21.3	21.6	5116	656
Heterotrophic dinoflagellates				
Oxyrrhis marina	26.6	12.8	2280	307
Crypthecodinium cf. *cohnii*	27.0	24.9	8720	1083
Phytoflagellates				
Cryptomonas sp.1	10.5	5.2	150	24
Cryptomonas sp.2	15.0	7.1	399	60
Eutreptiella marina	28.4	10.5	1646	226
Flagellate 6 μm	4.8	6.1	74	12
Diatoms				
Pennate 15 μm	15	3	71	9
Pennate 50 μm	50	6	942	74
Pennate 100 μm	100	10	4710	274
Cylindrotheca closterium	31.5	4.3	305	30
Minidiscus sp.1	10.8	12.7	1158	88
Minidiscus sp.2	5.5	7.1	169	18
Licmophora sp.	35.7	10.7	4583	268
Striatela unipunctata	67.4	57.2	45392	1723
Guinardia striata	96.9	29.3	65242	2312
Odontella sinensis	163.0	108.6	277906	7488
Ciliates				
Myrionecta rubra	18	16	2702	360
Strombidium sp.4	18.0	15.6	2642	353
Strombidium sp.6	40.2	24.5	10313	1268

Year 2000

Cryptomonas sp. was among the most abundant species in the lagoon during both years. Although this species is an important component of the phytoplankton community, it does not contribute greatly to the phytoplankton carbon, due to its small size (Table 3.2).

Larger species (over a 100 μm long) however, that are not important in terms of cell abundance, contributed greatly to the carbon biomass, e.g. some diatoms observed mainly at stations 6 and 7. Some species, such as *E. marina* and *C.* cf. *cohnii* were less abundant than *Cryptomonas* sp., but due to their larger size, they contributed significantly to the total carbon (Table 3.2).

High phytoplankton biomass was observed during 2000 in the Fleet, mainly due to the *P. micans* bloom, from August to October. During 2001, considerably lower phytoplankton biomass was recorded. *P. micans* is a thecate dinoflagellate possessing a theca rich in cellulose (carbon dense) and with cells from the Fleet typically measuring 30 μm long and 19 μm wide. Among the more abundant species in the Fleet, *P. micans* had the highest carbon content and was the principal contributor to the phytoplankton biomass in 2000.

At station 1, phytoplankton carbon varied between 510 μg C L^{-1} on 14/6/00, and 63936 μg C L^{-1} on 19/9/00 during 2000 (Fig. 3.22a). The first peak of carbon biomass on 1/6/00 was composed mainly by *C.* cf. *cohnii*, *E. marina* and *Cryptomonas* sp., while the second peak on 19/9/00 was represented by high biomass of *P. micans*.

At stations 2 and 3 the first peak of carbon biomass was observed on 11/7/00 and was composed of *E. marina* and the athecate species of 20 and 30 μm, and by *P. micans*, *C.* cf. *cohnii*, *O. marina* and *E. marina*, while later in the year the main peak in carbon content is due to the *P. micans* bloom (Fig. 3.22b, c).

Apart from *P. micans*, *E. marina*, the athecate of 20 μm, the athecate of 30 μm and *Scrippsiella* sp. were the main contributors to the phytoplankton carbon at station 4 (Fig. 3.22d). At station 5, *E. marina*, *O. marina* and *Scrippsiella* sp. represented most of the biomass in July, followed by higher *P. micans* biomass in October (Fig. 3.22e).

At station 6, the thecate dinoflagellate observed on 14/6/00 represented nearly 90% of the phytoplankton biomass on this date. *E. marina* and *P. micans* were other important species in terms of biomass (Fig. 3.22f).

At the marine end of the lagoon (station 7), large diatoms such as *Guinardia striata* and smaller ones like *Licmophora* sp. were important contributors to the total biomass. *E. marina* and *K.* cf. *mikimotoi* were important on 11/7/00 and 3/10/00 respectively, while *P. micans* represented 100% of the biomass on 4/11/00, when the bloom was washed out of the lagoon (Fig. 3.22g).

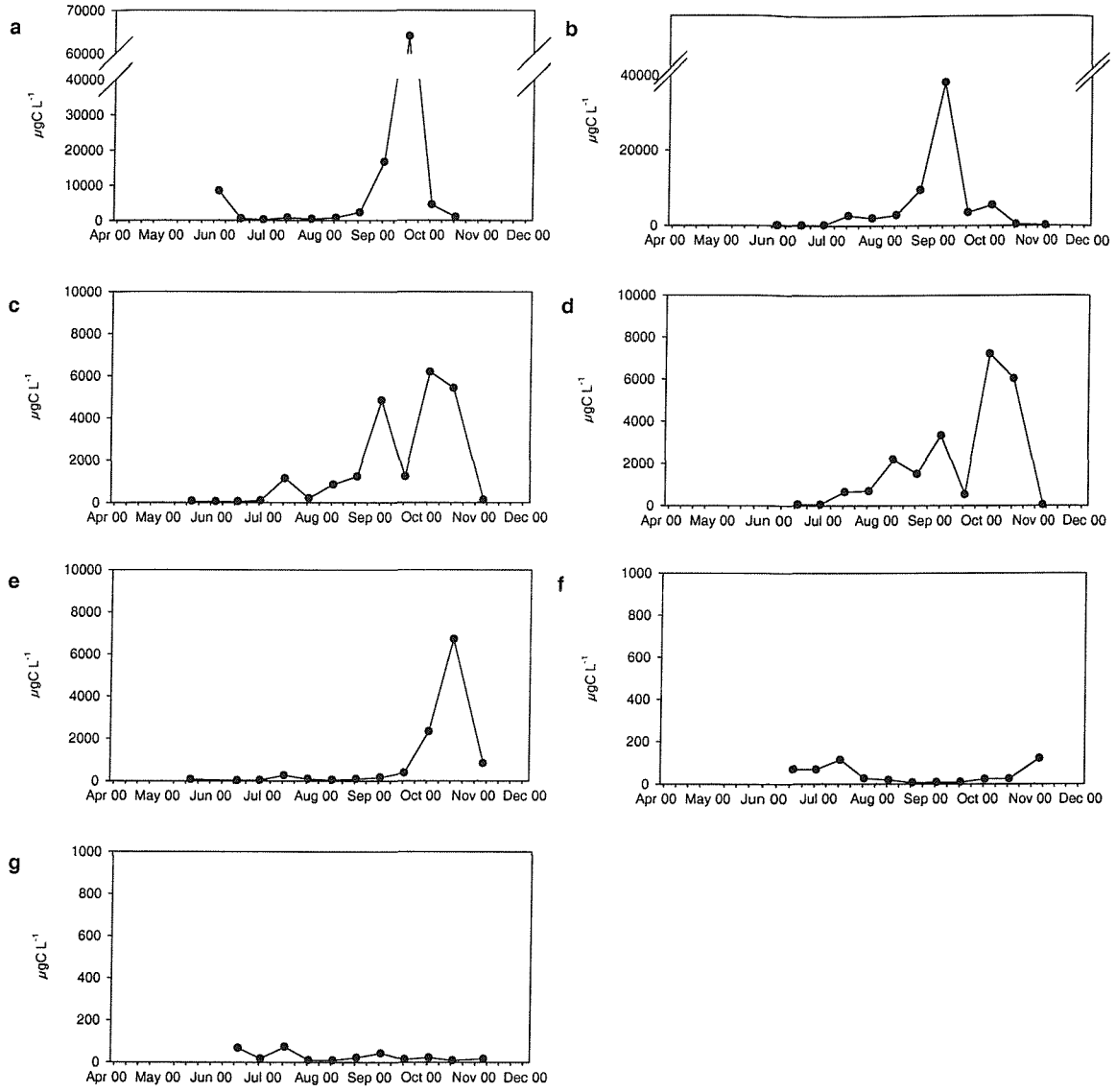


Figure 3.22: Phytoplankton total carbon content at stations 1 to 7 (a to g) in the Fleet lagoon during 2000. Note change of y scale on figures f and g.

Year 2001

During 2001 at station 1, *Cryptomonas* sp. represented 100 % of the biomass on 6/4/01. On 23/5/01 *C.* cf. *cohnii* and *M. rubra* dominated. *C.* cf. *cohnii* was the main species from 23/5/01 to 3/9/01 (Fig. 3.23a), and this was a long period when the heterotrophic biomass surpassed the autotrophic. On 3/9/01, an increase in the biomass of *Scrippsiella* sp. and the small diatom *Minidiscus* sp. was observed. *E. marina* and *C.* cf. *cohnii* interchanged as the dominant species in terms of biomass between late September and November (Fig. 3.23a).

At station 2, from 6/4/01 to 14/6/01 *Cryptomonas* sp. was the main contributor to the carbon biomass. *C.* cf. *cohnii* was a small component during this time and became

more important on 24/7/01, together with *Scrippsiella* sp. *M. rubra* followed as the main species in terms of biomass on 14/8/01 (Fig. 3.23b). From late September to November lower phytoplankton biomass was observed and the phytoflagellates were the main autotrophs, while the heterotrophic dinoflagellates *O. marina* and *C. cohnii* were also present.

At station 3, *Cryptomonas* sp. and sp. 2 were followed by *Scrippsiella* sp. as the dominant species on 14/6/01. On 24/7/01 and 14/8/01 *G. instriatum* became the dominant species and *Scrippsiella* sp. was still quite important. *M. rubra* followed as the main species in terms of biomass on 25/9/01 and it is possible that these cells were transported from station 2, as high biomass was observed at that station in August. October and November were characterized by low phytoplankton biomass at station 3 (Fig. 3.23c).

Stations 4, 5 and 6 showed relatively low carbon biomass during 2001, ranging from 3 to 187 μg C L^{-1}. *Scrippsiella* sp. and *M. rubra* were important species at stations 4 and 5, and at this later station, a more diverse assemblage was observed (Fig. 3.23d, e). The ciliate *Strombidium* sp. 4, *P. micans* and the small athecate dinoflagellate (11 μm) were also important components of the biomass. At station 6, *Cryptomonas* sp. and *C. closterium* shared the dominance on 6/4/01, while *M. rubra*, *Strombidium* sp. 4 and the athecate of 20 μm were the main components of the biomass on 14/6/01. *Guinardia striata* was important on 24/7/01, *M. rubra* on 14/8/01 and after that low biomass was observed with the total less than 9 μg C L^{-1} (Fig. 3.23f).

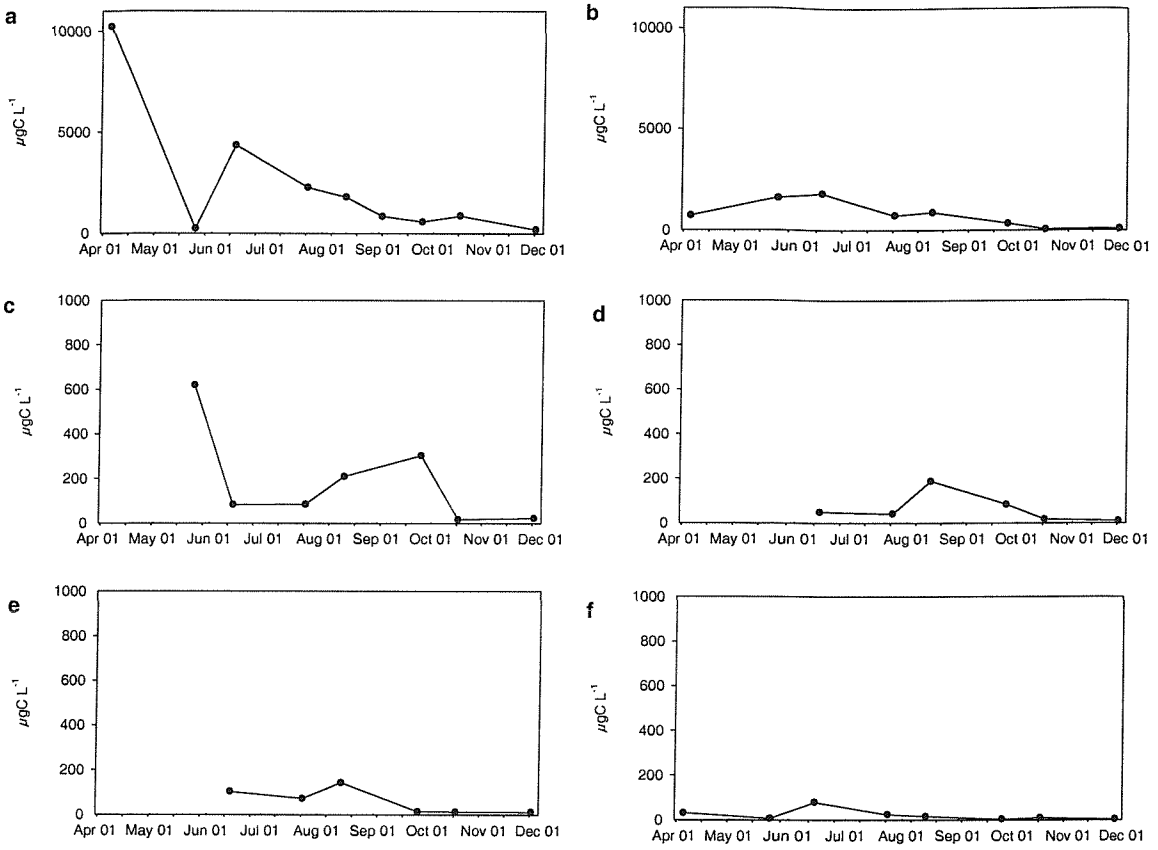


Figure 3.23: Phytoplankton total carbon content at stations 1 to 6 (a to f) in the Fleet lagoon during 2001.

3.2.8 Autotrophic carbon to chlorophyll-a ratio

The autotrophic carbon to chlorophyll-a ratio varied between 4 and 527 during 2000 and between 0.1 and 489 during 2001. The highest values observed during 2000 were associated with the *P. micans* bloom at stations 1 to 5 and particularly in August at station 2 and 3 (Fig. 3.24). These values were higher than the value of the slope from the plot of POC and chl-a (150) (Fig. 3.17). This might suggest that the carbon content of *P. micans* was overestimated. However, carbon content of *P. micans* from the Fleet lagoon was low relative to values described by Menden-Deur and Lessard (2000), who reported 2735 pg C $cell^{-1}$, compared to the value of 1428 pg C $cell^{-1}$ calculated in the present work.

The bloom of cryptophytes presented a lower C:chl-a ratio compared to *P. micans*. In 2001 the highest values of C:chl-a ratio were observed at station 2 in July and were associated with high biomass of *Scrippsiella* sp. and cryptophytes.

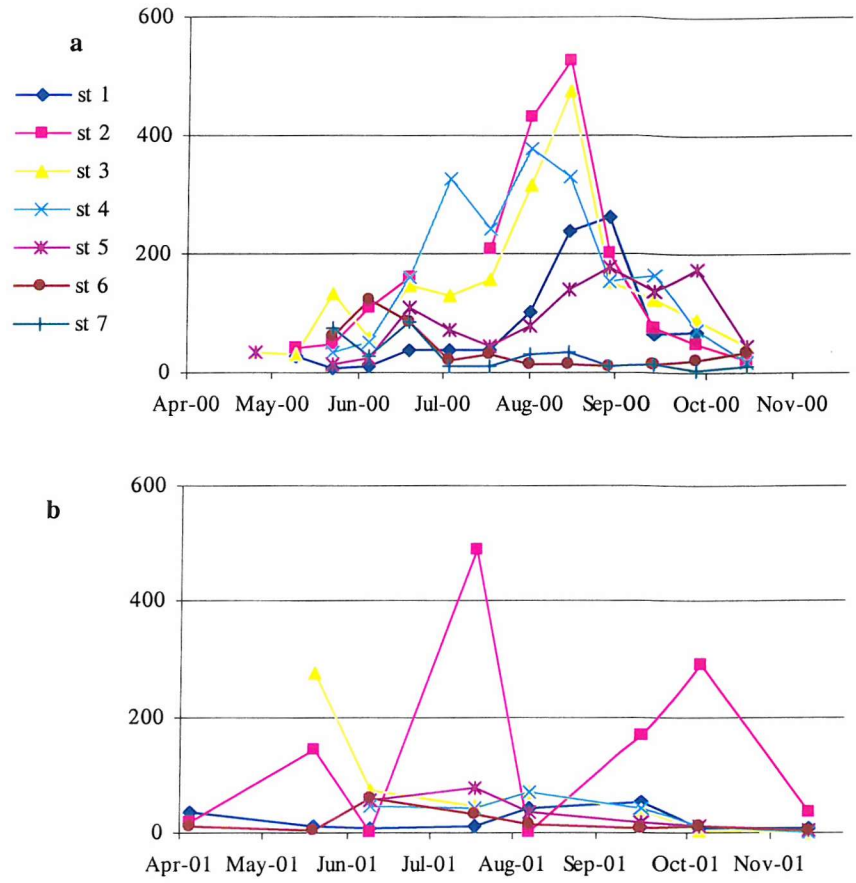


Figure 3.24: Autotrophic carbon to chl-a ratio during 2000 (a) and 2001 (b).

3.2.9 Dinoflagellate cysts in the sediment

Table 3.3 shows the results of quantifying dinoflagellate cyst abundances in sediments collected from the Fleet. The most numerous cyst found in the lagoon was that of *Scrippsiella rotunda* (Fig. 3.25). Higher cyst abundances were observed at station 2, although cysts of this species were also observed at stations 1, 3 and 4. The temporal distribution of *S. rotunda* cysts in sediments from the Fleet showed some variability, that may be partly due to the fact that samples were not always collected exactly from the same place at that station. However, temporal changes observed were quite significant, and may be related to cyst germination or to deposition of cysts from *S. rotunda* motile stages. It is likely that the large increase in cyst abundances observed from 23/5/01 to 14/6/01 (Table 3.3) was not real, but related to heterogeneous spatial (patchy) distribution of cysts in sediments at station 2. If this increase have been caused by encystment of *S. rotunda*, cells of this species should have been found in the plankton on 23/5/01.

Figure 3.25: A *Scrippsiella rotunda* cyst under 400x magnification from a sediment sample from Cloud's Hill, Fleet lagoon.

Scrippsiella sp. was observed in the water column at station 2 from 11/7/00 to 19/9/00 and on 24/7/01, while at stations 1 and 3 the species was also observed on 14/6 2000 and 2001. The decrease in cyst abundances observed between 14/6/01 and 24/7/01 at station 2 (Table 3.3) is more likely to have been caused by the germination of *S. rotunda* cysts from the sediment, resulting in the *Scrippsiella* population observed in the water column on 24/7/01. Cyst abundances were similar in July and August, but increased considerably on 25/9/01 (Table 3.3), suggesting that *Scrippsiella* cells have encysted and settled down to bottom sediments by this date. This is supported by the fact that *Scrippsiella* was not found on the water column later in the year.

Table 3.3: *Scrippsiella rotunda* cyst abundances in the top 3 cm of sediment samples from the Fleet lagoon.

Date	Station	*Scrippsiella rotunda* cyst g sediment^{-1}
23/5/01	2	187
14/6/01	2	832
24/7/01	2	261
14/8/01	2	223
25/9/01	2	1075
27/11/01	1	29
27/11/01	2	248
27/11/01	3	68
27/11/01	4	18
27/11/01	5	0
27/11/01	6	0

Another cyst found in sediments from stations 2 and 3 was a round, small cyst that appeared similar to the cyst of *Alexandrium minutum*. It was much less abundant than *S. rotunda* cyst and only 19 and 23 cysts were found per gram of sediment at stations 2 and 3 on 27/11/00. The cysts were identified based upon descriptions in the literature, but it is recognised that exact identification requires the hatching and identification of the motile stage.

3.2.10 Meteorological data

Meteorological data was obtained either from the Portland met station (50° 33.9' N, 2° 26.9' W) (rainfall and wind) or from the Thorney Island met station (50° 49.0' N, 0° 58.2' W) the closest station to the Fleet with available radiation measurements. River flow data was obtained for the river Wey at Broadwey to provide an indication of seasonal variation of river flow in the region, as the small streams that drain into the Fleet are not gauged.

The photosynthetic active radiation (PAR) peaked in June in both years and showed a typical seasonal pattern with low values observed during the winter (Fig. 3.26).

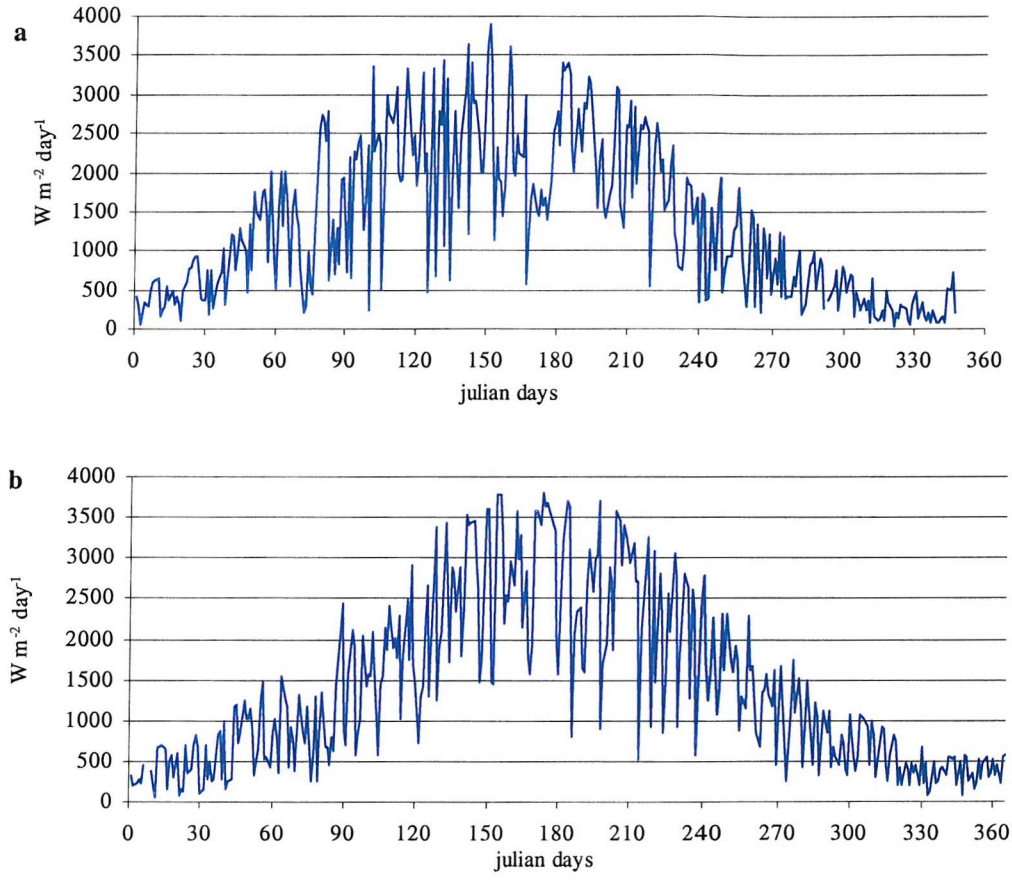


Figure 3.26: Photosynthetic active radiation (PAR) at the Thorney Island meteorological station during 2000 (a) and 2001 (b).

Rainfall also showed a clear seasonal pattern with lower values in the summer, although high interannual variability was observed among the winter months of both years (Fig. 3.27).

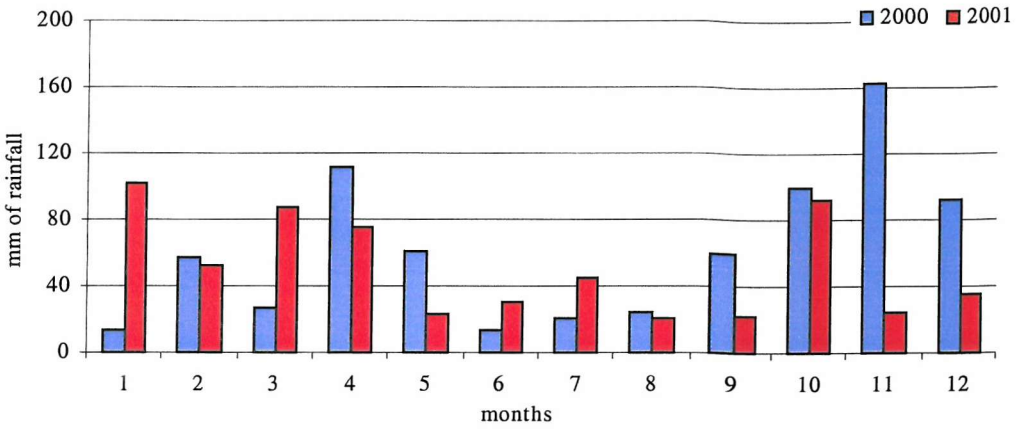


Figure 3.27: Monthly total rainfall at Portland meteorological station during 2000 and 2001.

The daily averaged wind was calculated for directions across the width (NE to SW) and along the length (SE to NW) of the Fleet lagoon and are shown on Fig. 3.28 and 3.29.

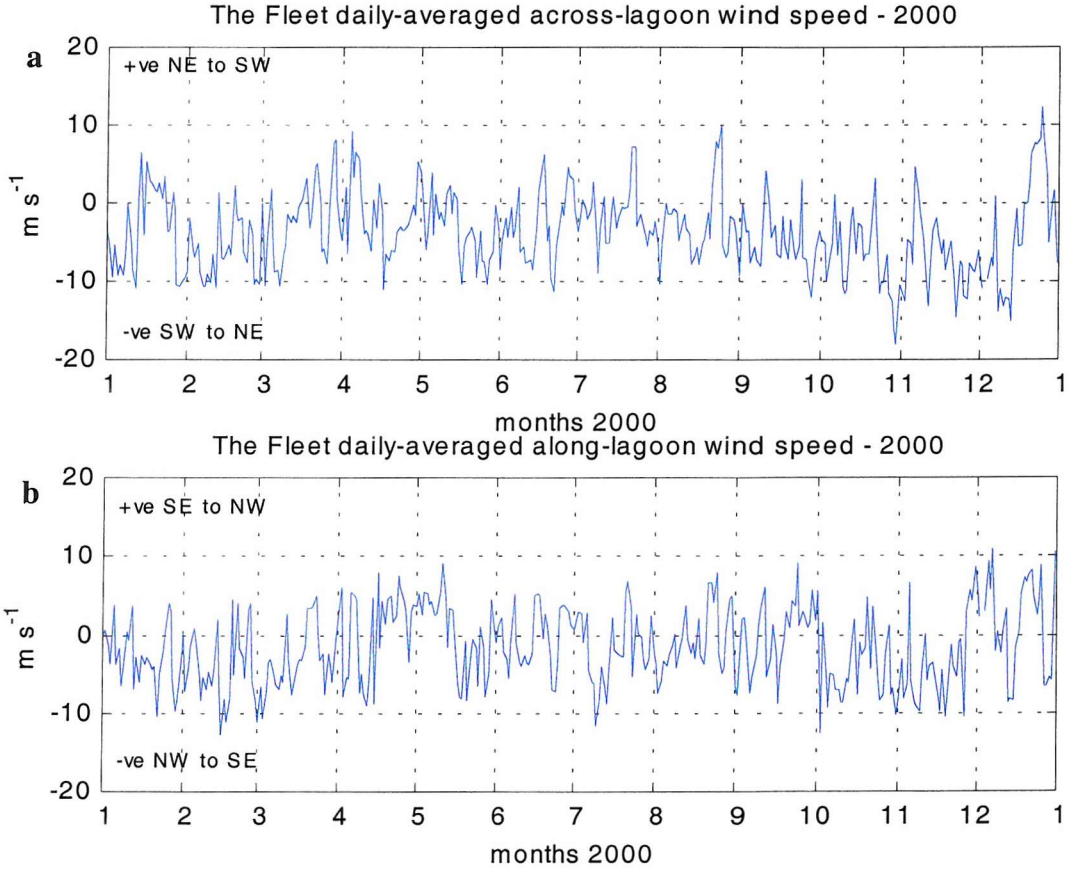


Figure 3.28: Daily-averaged (a) across lagoon and (b) along lagoon wind speed during 2000.

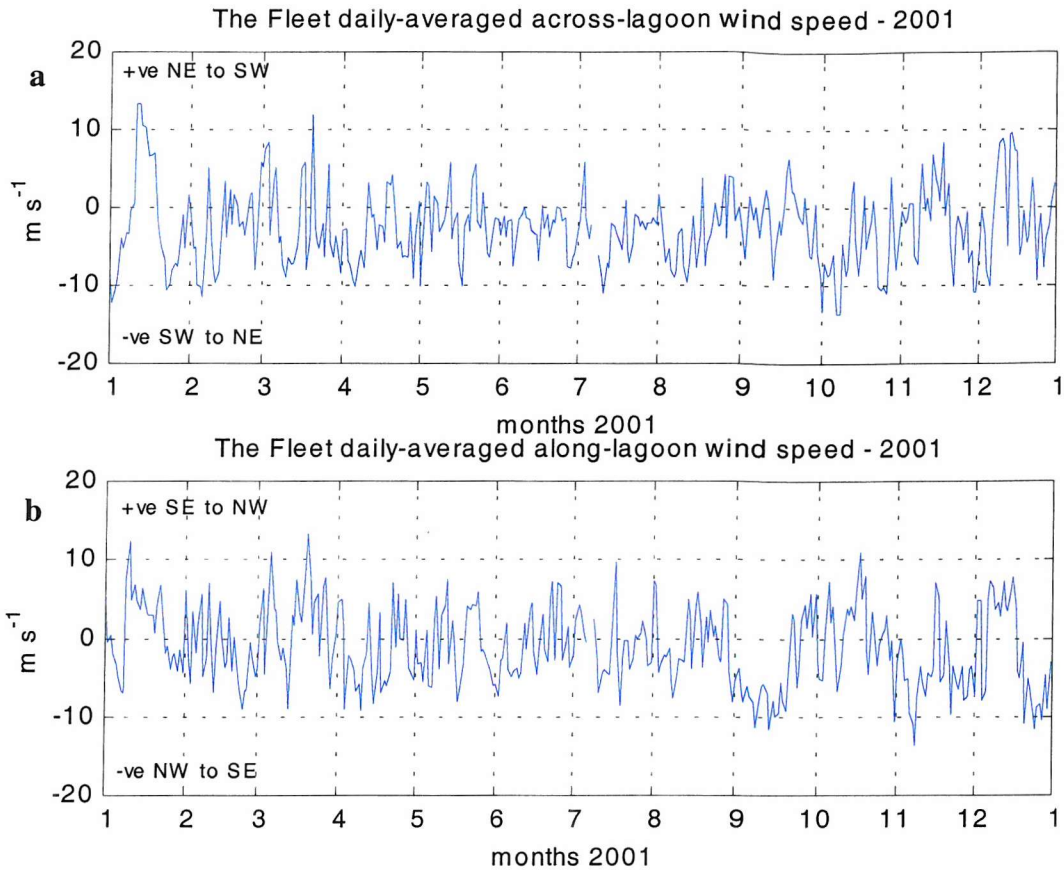


Figure 3.29: Daily-averaged (a) across lagoon and (b) along lagoon wind speed during 2001.

River flow showed a pattern consistent with the rainfall, of lower river discharge during the summer months (Fig. 3.30). However, as observed for the rainfall data, there was high interannual variability between the period of October to December in these two years. In general, 2000 and 2001 showed increased river flow, with monthly means higher than average flows for these months over the last 26 years; particularly during the period between October 2000 to April 2001, when river flow was extremely high due to high rainfall in this period.

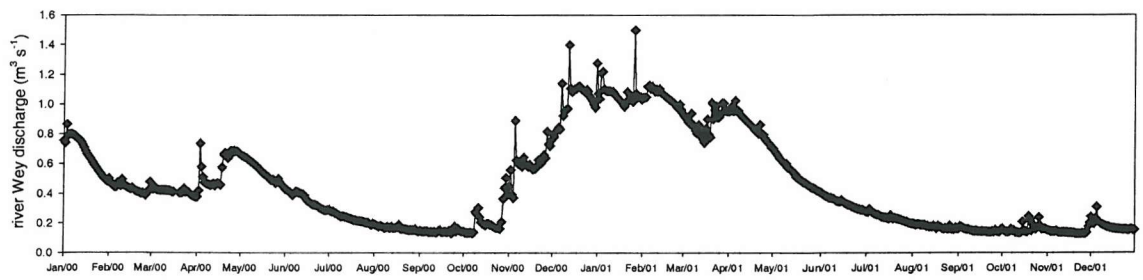


Figure 3.30: River Wey daily flow ($m^3 s^{-1}$) during 2000 and 2001.

3.2.11 Multivariate Analysis

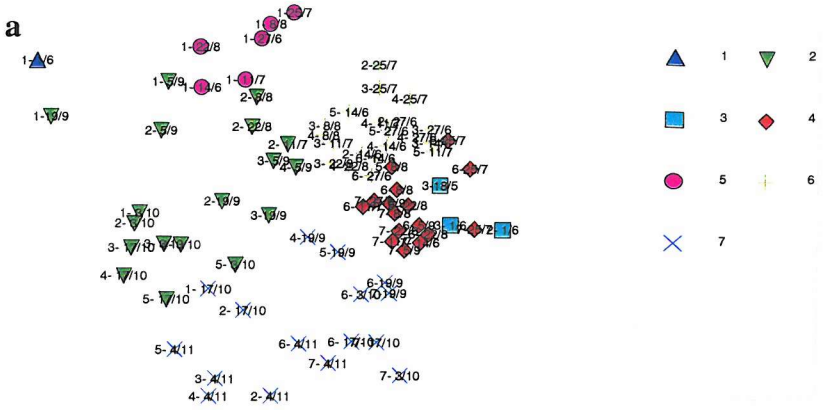
Multivariate analysis provides statistical methods for the study of the joint relationships of variables in data that contain intercorrelations. The patterns of relationships among samples can be described by ordination (reduction of a matrix of distances or similarities among samples to a few dimensions in a non-metric multidimensional scaling, MDS), or by cluster analysis (classification of samples into hierarchical categories on the basis of a matrix of inter-sample similarities) (James and McCulloch, 1990). MDS was used to identify any temporal pattern of plankton community change in the Fleet lagoon.

Year 2000

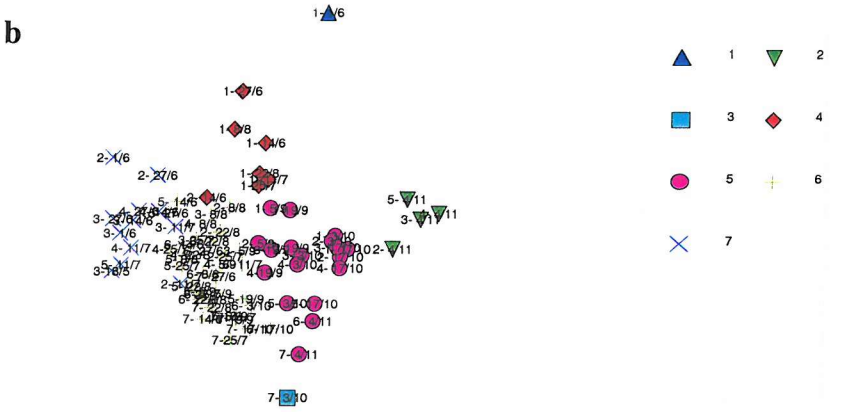
Environmental data

Cluster and MDS analysis of environmental data are based on a normalised Euclidean distance with previous log transformation of the data. Two types of analysis have been carried out. MDS1, including the variables salinity, temperature, pH, phosphate, silicate, chlorophyll-a, particulate organic carbon (POC), particulate organic nitrogen (PON), total rainfall in the two weeks preceding the sampling date and average PAR in the week before the sampling date (Fig. 3.31a). MDS2 was performed using the parameter combination with the highest rank correlation ($\rho = 0.46$) value from the BIOENV routine of PRIMER, this calculates a measure of agreement between two similarity matrices, the fixed Bray-Curtis similarity biotic matrix (phytoplankton species) and each of the possible normalised Euclidean distance abiotic matrices (with different combinations of selected abiotic parameters). The BIOENV routine chooses the environmental combination "best matching" the biotic pattern, in this case: salinity, temperature, pH and silicate concentrations (Fig. 3.31b).

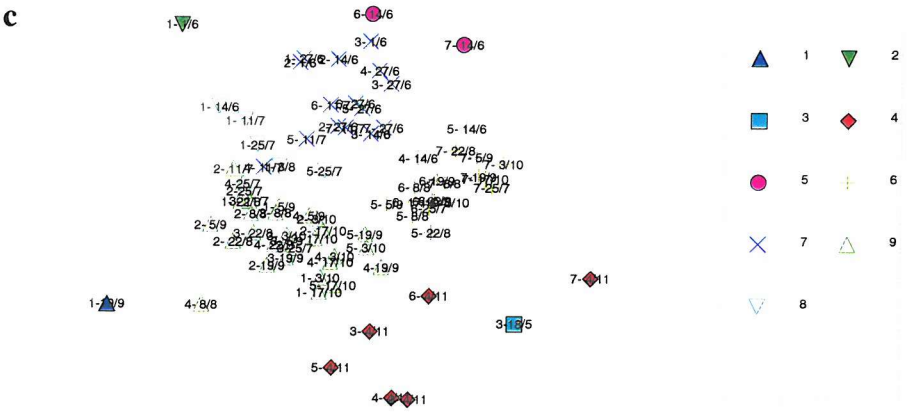
The stress of MDS1 was 0.12 and MDS2 was 0.11, corresponding to a good ordination with no real prospect of a misleading interpretation (Clarke and Warwick, 1994). Cluster analysis was performed using the two distinct combinations of variables as in MDS1 and MDS2. Cluster1 and cluster2 were superimposed on the MDS plots using different markers and colours and reinforce the MDS ordinations (Fig. 3.31a, b). The cluster1 dendogram shows separation at a normalised Euclidean distance of 4 into seven major groups (Fig. 3.32a). Further separation into sub-groups occurs at a normalised Euclidean distance of 3 and leads to eleven groups. Cluster2 shows separation at a normalised distance of 2.3 into seven groups (Fig. 3.32b).



Stress: 0.12



Stress: 0.11

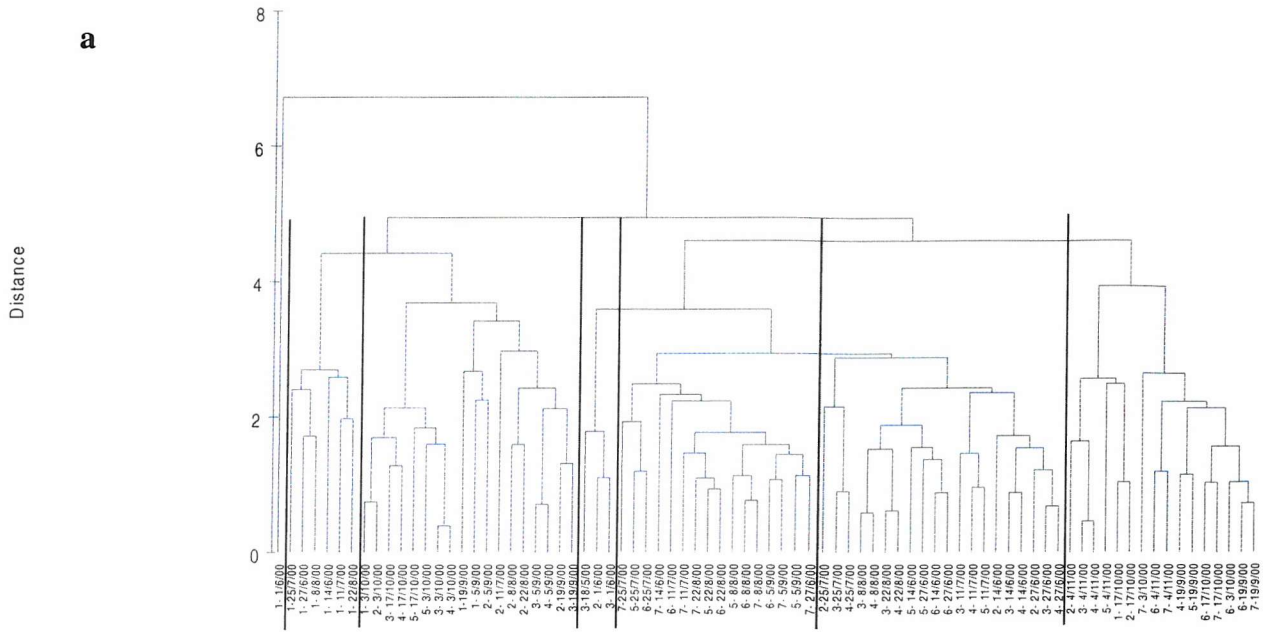


Stress: 0.21

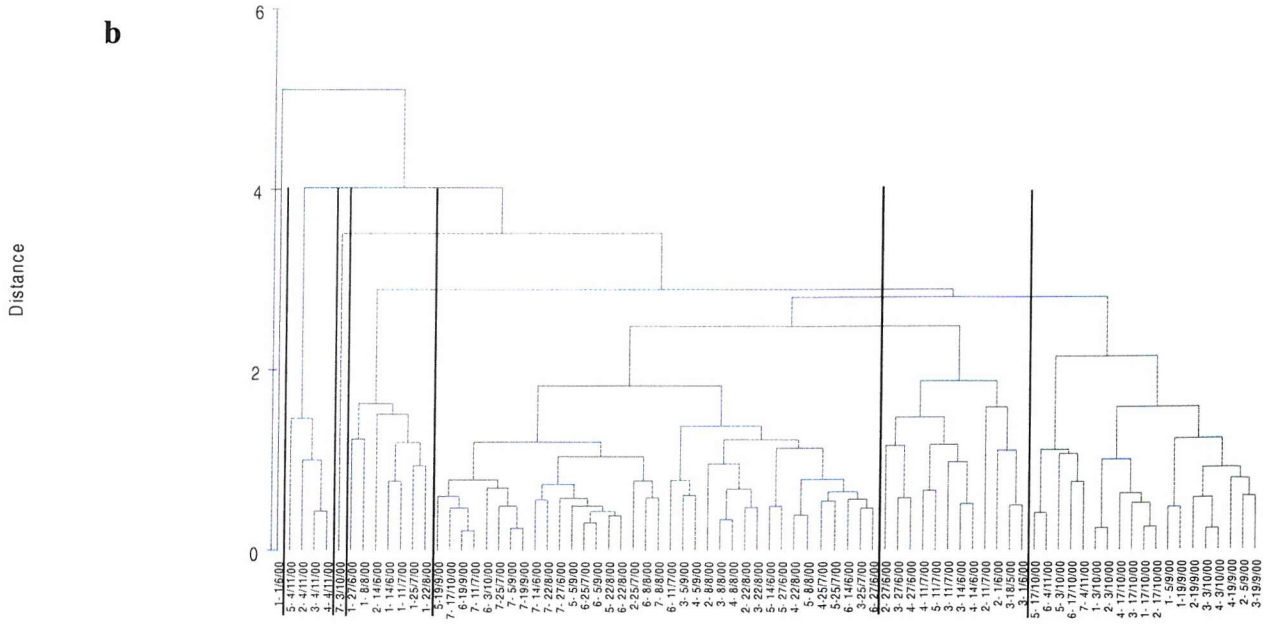
Figure 3.31: Non-metric multidimensional scaling ordination of 2000 data including (a) salinity, temperature, pH, phosphate, silicate, chlorophyll-a, POC, PON, rainfall and PAR

data (MDS1), (b) salinity, temperature, pH and silicate only (MDS2) and (c) phytoplankton abundance (MDS3). The legend refers to cluster groups superimposed on the MDS. The first number of each marker refers to the station number, followed by the date.

a



b



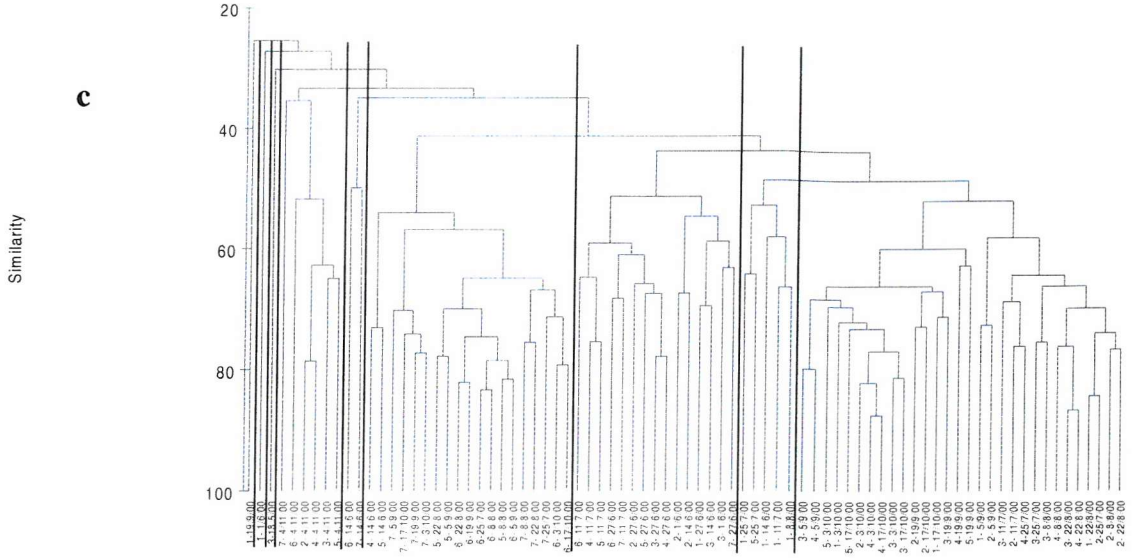


Figure 3.32: Dendogram of cluster analysis of 2000 dataset, including (a) salinity, temperature, pH, phosphate, silicate, chlorophyll-a, POC, PON, rainfall and PAR data (cluster1), (b) salinity, temperature, pH and silicate only (cluster2) and (c) phytoplankton abundance (cluster3). The first number of each marker refers to the station number, followed by the date.

These results indicate high dissimilarity of environmental parameters at station 1 from all other stations from June to August 2000 (group 5 of cluster1, Fig. 3.32.a and group 4 of cluster2, Fig. 3.32b) and high dissimilarity of samples collected on 4/11/00 at stations 2 to 5, considering salinity, temperature, pH and silicate data, as those samples were grouped together in group 2 of cluster 2 (Fig. 3.32b). Indeed, a clear seasonal pattern was observed of cluster2. Group 4 includes summer samples at station 1 (14/6 to 22/8/00, plus st. 2 on 14/6/00). Group 7 corresponds to samples from stations 2, 3 and 4 in early-summer (st. 2 1/6/00, 27/6/00 and 11/7/00, st. 3 18/5-11/7/00, st. 4 14/6-11/7), while group 6 includes samples of these stations during late-summer (st.2 25/7-22/8/00 and st. 3 and 4 25/7-5/9/00) as well as samples from stations 6 and 7 throughout summer and autumn (st. 6 14/6-3/10/00, st. 7 14/6-17/10) and a few samples from station 5 (14-27/6/00, 25/7-19/9/00). Group 5 of cluster2 is composed of samples from autumn at stations 1 to 4 (st.1 and 2, 5/9-17/10/00, st.3 and 4, 19/9-17/10/00), "winter" conditions at stations 6 and 7 (st. 6 17/10-4/11/00, st.7 4/11/00) and few samples from station 5 during autumn (3-17/10/00). Group 2 refers to "winter" conditions at stations 2 to 5 (no data from st. 1).

This analysis indicates that during summer 2000, the Fleet lagoon was spatially more heterogeneous, with the Abbotsbury embayment showing consistently lower salinity

and higher silicate concentrations than stations 2 to 4, due to the more important freshwater contribution to this region. pH values were also lower at station 1, compared to stations 2 to 4 throughout June and July. It is likely that the elevated pH values in the mid Fleet are caused by seaweed and *Zostera* growth and photosynthetic activity, during this time. The distinction between early and late summer in samples from the mid Fleet is probably due to a marked increase in salinity and temperature at stations 2-4 towards late July. The autumn season was marked by increased rainfall from mid September 2000, causing an enhanced freshwater inflow into the lagoon that horizontally mixed the waters of the Fleet, turning them spatially more homogeneous in respect to salinity, temperature, pH and silicate concentrations. Salinity and temperature were considerably lower on 4/11/00 at stations 2 to 4, and silicate concentrations were extremely high and similar at these stations, showing high dissimilarity from the autumn samples, indicating that these samples may characterise "winter" conditions in the Fleet lagoon, although an extended survey would be necessary to confirm this. At stations 6 and 7 the seasonal cycle was not as marked as at other stations, due to its marine character. Station 5 is located in a shallow area with eelgrass beds dominated by *Zostera marina*, it is clearly a transitional zone, still under strong tidal and marine influence. This is likely to be the reason why samples from this station did not follow consistently the seasonal pattern observed at other stations.

Phytoplankton

Cluster and MDS analysis of phytoplankton species abundances are based on Bray-Curtis similarities. The stress of MDS3 was 0.21 (Fig. 3.31c), which indicates a potential useful 2-dimensional picture, though too much reliance should not be placed on the detail of the plot, and a cross-check of any conclusions should be made against those from an alternative technique, like the superimposition of cluster groups (Clarke and Warwick, 1994). Nine species groups can be identified at 55 % Bray-Curtis similarity level on cluster3 (Fig. 3.32c). Three groups are composed of only one sample, group 1 (1-19/9/00), group 2 (1-1/6/00) and group 3 (3-18/5/00) and correspond to samples where a single species was dominant, independently of its absolute abundance. *P. micans* constitutes 92% of the total cell density of sample 1-19/9/00 (group 1), *Cryptomonas* sp. corresponds to 73% of the total abundance in sample 1-1/6/00 (group 2), while a pennate diatom (average length 50 μm) represents 67% of the total abundance in sample 3-18/5/00 (group 3). Sample 1-1/6/00 was also separated into a distinct group when only salinity, temperature, pH and silicate data was used in the analysis, on cluster2 (Fig. 3.32b, group 1).

Group 5 is composed of two samples (6- and 7-14/6/00) with very similar mean *Cryptomonas* sp. and pennate 50 μm abundances. However, these two samples were far apart on the 3D MDS3 plot (data not shown), and group 5 will not be discussed here.

Group 4 represents samples from stations 2 to 7 on 4/11/00 (station 1 was not sampled on this date). Similar to the result of cluster2 (salinity, temperature, pH and silicate data), phytoplankton data reinforces the separation of November samples into a distinct group, the "winter" samples (Fig. 3.32c). Group 6 of cluster3 is mainly composed of samples from stations 6 and 7 from 25/7/00 to 17/10/00, although samples from station 5 on 14/6/00, 8/8/00, 22/8/00 and 5/9/00 and from station 4 on 14/6/00 were also included in this group, indicating a distinct species composition at this end of the lagoon (grouping similar to group 6 of cluster2). Group 7 is composed of samples from stations 2 and 3 on 1/6/00 and 14/6/00, stations 1 to 7 on 27/6/00 and stations 4 to 7 on 11/7/00. Group 8 represents samples from station 1 on 14/6/00, 11/7/00, 25/7/00 and 8/8/00 and station 5 on 25/7/00 (similar to group 4 of cluster2), while group 9 is represented by samples from stations 2 and 3 on 11/7/00, 2 to 4 on 25/7/00 and 8/8/00, 1 to 4 on 22/8/00 and 5/9/00, 2 to 5 on 19/9/00, 1 to 5 on 3 and 17/10/00. Therefore cluster analysis distinguished species assemblages that varied both temporally and spatially along the lagoon (Table 3.4).

Table 3.4: Summary of main species contributing to the similarity between samples from each group of cluster3, showing average abundances in brackets. Cumulative contributions of species listed in groups 1 to 9 are respectively 92, 73, 67, 76, 81, 54, 63, 60 and 56%. See appendix 12 for a graphic representation of how cluster groups are distributed with respect to sampling date and station.

Date	St.	Group	Main species (avg cells mL^{-1})
18/5/00	3	3	Pennate 50 µm (127)
1/6/00	1	2	*Cryptomonas* sp. (52170)
	2, 3	7	*Cryptomonas* sp. (424), Pennate 50 µm (83), *M. rubra* (7), *Scrippsiella* sp. (9)
14/6/00	1	8	*Cryptomonas* sp. (1117), *E. marina* (436), *C.* cf. *cohnii* (219), *M. rubra* (23)
	2, 3	7	*Cryptomonas* sp. (424), Pennate 50 µm (83), *M. rubra* (7), *Scrippsiella* sp. (9)
	4, 5	6	*Cryptomonas* sp. (60), Pennate 50 µm (10), Pennate 15 µm (7), flagellates 6 µm (17), *Strombidium* sp. 4 (11)
	6, 7	5	*Cryptomonas* sp. (31), Pennate 50 µm (31), *M. rubra* (24)
27/6/00	1 to 7	7	*Cryptomonas* sp. (424), Pennate 50 µm (83), *M. rubra* (7), *Scrippsiella* sp. (9)
11/7/00	1	8	*Cryptomonas* sp. (1117), *E. marina* (436), *C.* cf. *cohnii* (219), *M. rubra* (23)
	2, 3	9	*P. micans* (2923), *Cryptomonas* sp. (375), flagellates 6 µm (112), Pennate 50 µm (43)
	4 to 7	7	*Cryptomonas* sp. (424), Pennate 50 µm (83), *M. rubra* (7), *Scrippsiella* sp. (9)
25/7/00	1, 5	8	*Cryptomonas* sp. (1117), *E. marina* (436), *C.* cf. *cohnii* (219), *M. rubra* (23)
	2 to 4	9	*P. micans* (2923), *Cryptomonas* sp. (375), flagellates 6 µm (112), Pennate 50 µm (43)
	6, 7	6	*Cryptomonas* sp. (60), Pennate 50 µm (10), Pennate 15 µm (7), flagellates 6 µm (17), *Strombidium* sp. 4 (11)
8/8/00	1	8	*Cryptomonas* sp. (1117), *E. marina* (436), *C.* cf. *cohnii* (219), *M. rubra* (23)
	2 to 4	9	*P. micans* (2923), *Cryptomonas* sp. (375), flagellates 6 µm (112), Pennate 50 µm (43)
	5 to 7	6	*Cryptomonas* sp. (60), Pennate 50 µm (10), Pennate 15 µm (7), flagellates 6 µm (17), *Strombidium* sp. 4 (11)
22/8/00 5/9/00	1 to 4	9	*P. micans* (2923), *Cryptomonas* sp. (375), flagellates 6 µm (112), Pennate 50 µm (43)
	5 to 7	6	*Cryptomonas* sp. (60), Pennate 50 µm (10), Pennate 15 µm (7), flagellates 6 µm (17), *Strombidium* sp. 4 (11)
19/9/00	1	1	*P. micans* (43623)
	2 to 5	9	*P. micans* (2923), *Cryptomonas* sp. (375), flagellates 6 µm (112), Pennate 50 µm (43)
	6, 7	6	*Cryptomonas* sp. (60), Pennate 50 µm (10), Pennate 15 µm (7), flagellates 6 µm (17), *Strombidium* sp. 4 (11)
3/10/00 17/10/00	1 to 5	9	*P. micans* (2923), *Cryptomonas* sp. (375), flagellates 6 µm (112), Pennate 50 µm (43)
	6, 7	6	*Cryptomonas* sp. (60), Pennate 50 µm (10), Pennate 15 µm (7), flagellates 6 µm (17), *Strombidium* sp. 4 (11)
4/11/00	2 to 7	4	*P. micans* (122), *M. rubra* (33), *Strombidium* sp. 1 (11)

Some temporal changes are interesting to note. Species from group 3, initially present at station 3 in May (the only data from May 2000 in the multivariate analysis is from station 3) were replaced by the species assemblage from group 7 during June at this station (Table 3.4). This species assemblage was later observed from station 1 to 7 on 27/6/00 and some species were probably flushed out from the lagoon between 11 and 25/7/00 (Table 3.4). Species of group 9 followed at stations 2 to 4 on 25/7/00 and 8/8/00, and by 22/8/00 and 5/9/00 this assemblage extended from station 1 to 4 (similar to group 6 of cluster2, late summer conditions). Group 9 is mainly composed of high *P. micans*

abundance, but by 19/9/00 *P. micans* numbers were so high at station 1 that this sample was separated into a distinctive group (1 of cluster3). From 19/9/00 and during October, the *P. micans* bloom (group 9 of cluster3) extended from station 1 to 5. It is noteworthy that group 9 of cluster3 can be further divided into two subgroups at approximately 50 % Bray Curtis similarity (dotted line in Fig. 3.32c), dividing samples from st.1 on 22/8-5/9/00, st. 2 on 11/7-5/9/00, st. 3 on 11/7-22/8/00 and st.4 on 25/7-22/8/00 from samples from these same stations from either 22/8 or 5/9/00 until 17/10/00. These sub-groups agree reasonably well with group 6 (late summer at stations 2 to 4) and group 5 (autumn at stations 1 to 4) of cluster2.

By 4/11/00 *P. micans* abundances had markedly decreased, although it was still the dominant species. However, ciliates were now observed associated with *P. micans*, and this new assemblage together with lower *P. micans* numbers characterized group 4 of cluster3, that corresponds to group 2 of cluster2 ("winter" conditions) (Table 3.4).

The species assemblage of group 8 was initially observed at station 1 on 14/6/00 and then from 11/7/00 to 8/8/00, a period when station 1 showed a distinct phytoplankton assemblage from elsewhere in the lagoon. By 22/8/00, the *P. micans* bloom reached station 1, although it is possible that the bloom was moving in and out of the lagoon (from stations 2 and 3 to stations 1 and 4) with tidal and wind forcing before this date.

At the other end of the lagoon, at stations 6 and 7 (and 5 from 8/8/00 to 5/9/00), species composing group 6 were observed from 25/7/00 to 17/10/00. This species assemblage was replaced by species of group 4 on 4/11/00.

Cryptomonas sp. was a remarkable contributor to several species groups. Some groups are differentiated by the species associated with *Cryptomonas* sp. and others by the actual mean abundance of this species, that decreases from group 2 to 8, 7, 9, 6 and 5. Interesting is the association of *C.* cf. *cohnii* and *M. rubra* with *Cryptomonas* sp. in group 8. *M. rubra* ingests cryptophytes and steals their organelles (Gustafson et al. 2000), and *C.* cf. *cohnii* is a heterotrophic dinoflagellate that feeds on unicellular red algae. Their co-occurrence suggests that grazing of cryptophytes by these two species is likely to be taking place.

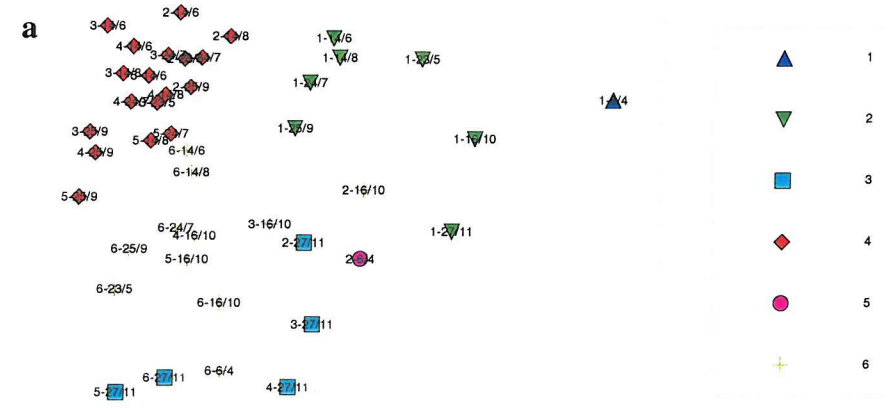
Noteworthy is the remarkable similarity between some groups of clusters 2 and 3, indicating that temporal and spatial changes of salinity, temperature, silicate and pH values were indeed accompanied by changes in phytoplankton species composition, as highlighted by the statistical analysis.

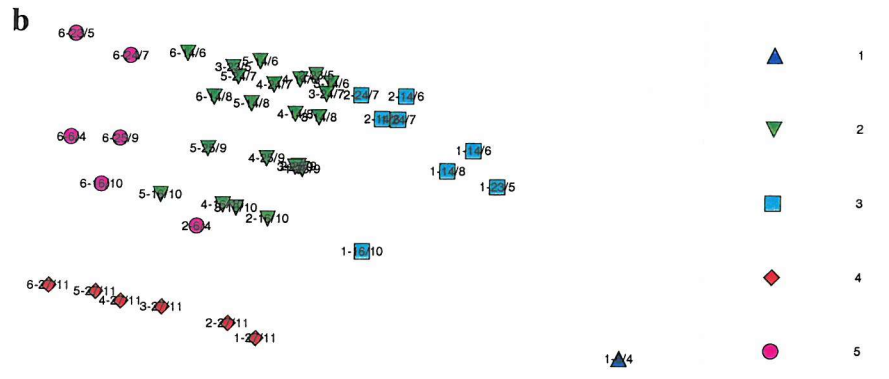
Year 2001

Environmental data

As for data from the year 2000, MDS and cluster analysis were performed for environmental data from the year 2001. Analysis were performed for all variables (MDS4 and cluster4) and using only the parameter combination with the highest rank correlation ($\rho = 0.56$) value from the BIOENV routine of PRIMER (MDS5 and cluster5). MDS4 included the following variables: salinity, temperature, pH, ammonium, phosphate, silicate, nitrate, N/P ratio, chlorophyll-a, total rainfall in the two weeks preceding the sampling date and average PAR in the week before the sampling date (Fig. 3.33a). MDS5 was performed using data for salinity, phosphate and average PAR in the week before the sampling date (Fig. 3.33b).

The stress of MDS4 was 0.14 and MDS5 was 0.07, indicating good representation of the data. The lower stress of MDS5 is likely to be due to the low level of the dimensional picture (3 variables only). Cluster analysis was performed using the two distinct combinations of variables as in MDS4 and MDS5. Cluster4 and cluster5 were superimposed on the MDS plots and reinforce the MDS ordinations. Cluster4 shows separation at a normalised Euclidean distance of just over 4 into six major groups (Fig. 3.34a). Cluster5 shows separation at a normalised distance of 2 into 5 groups (Fig. 3.34b).





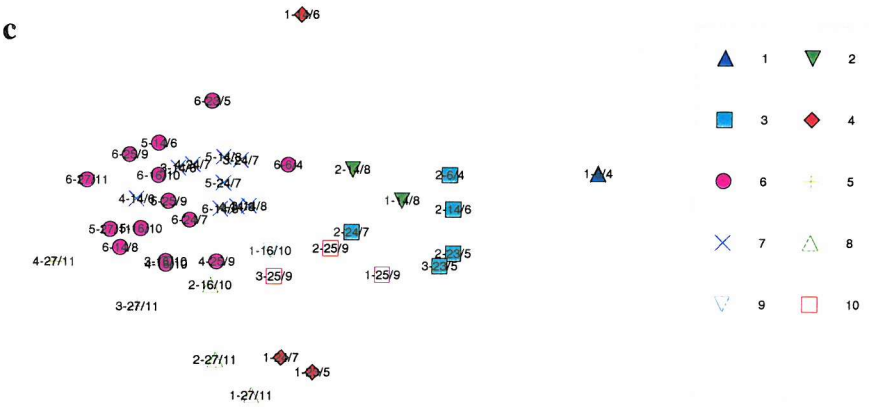
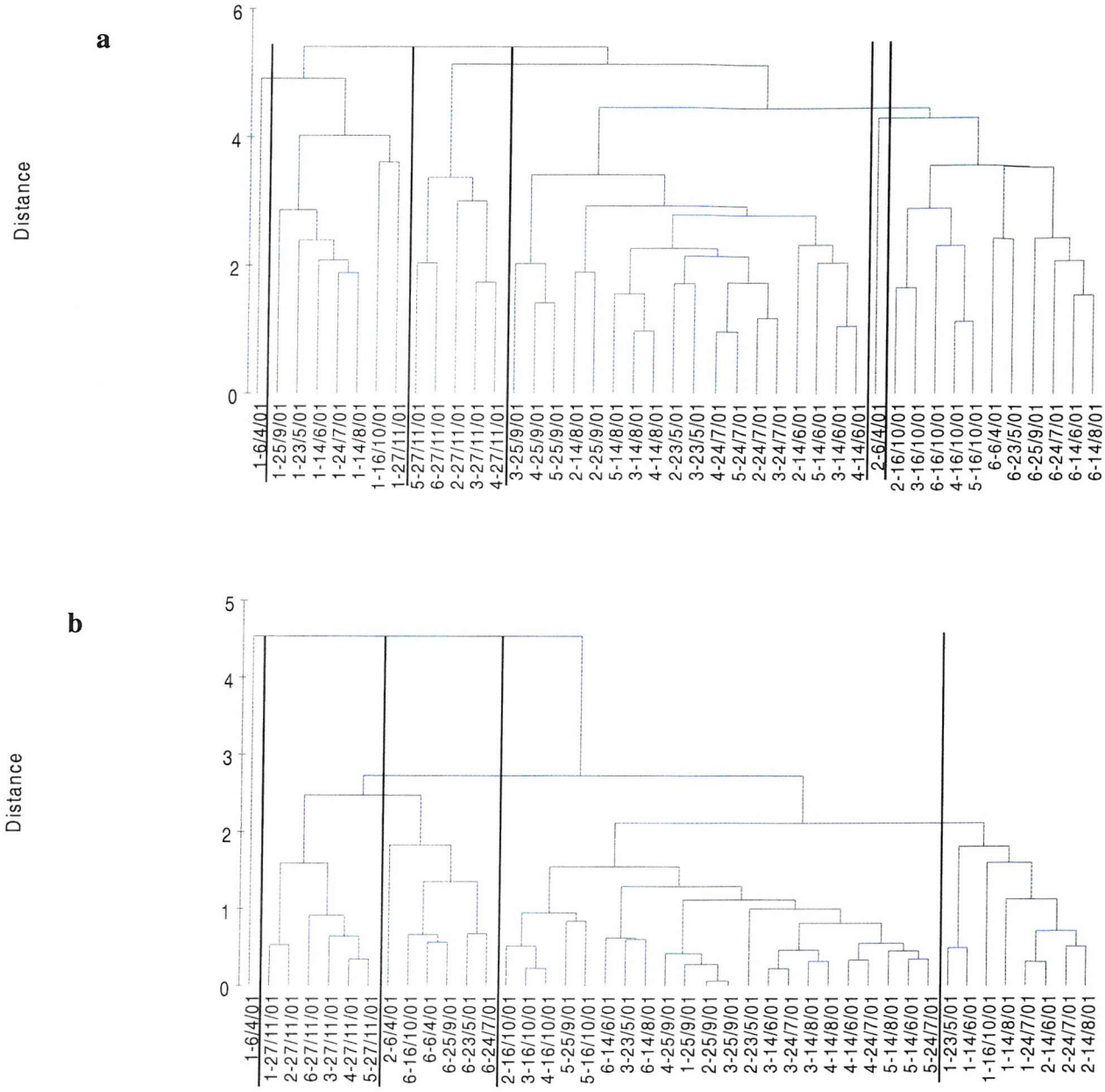


Figure 3.33: Non-metric multidimensional scaling ordination of 2001 data including (a) salinity, temperature, pH, ammonium, phosphate, silicate, nitrate, N/P ratio, chlorophyll-a,

rainfall and PAR data (MDS4), (b) salinity, phosphate and average PAR only (MDS5) and (c) phytoplankton abundance (MDS6). The legend refers to cluster groups superimposed on the MDS. The first number of each marker refers to the station number, followed by the date.



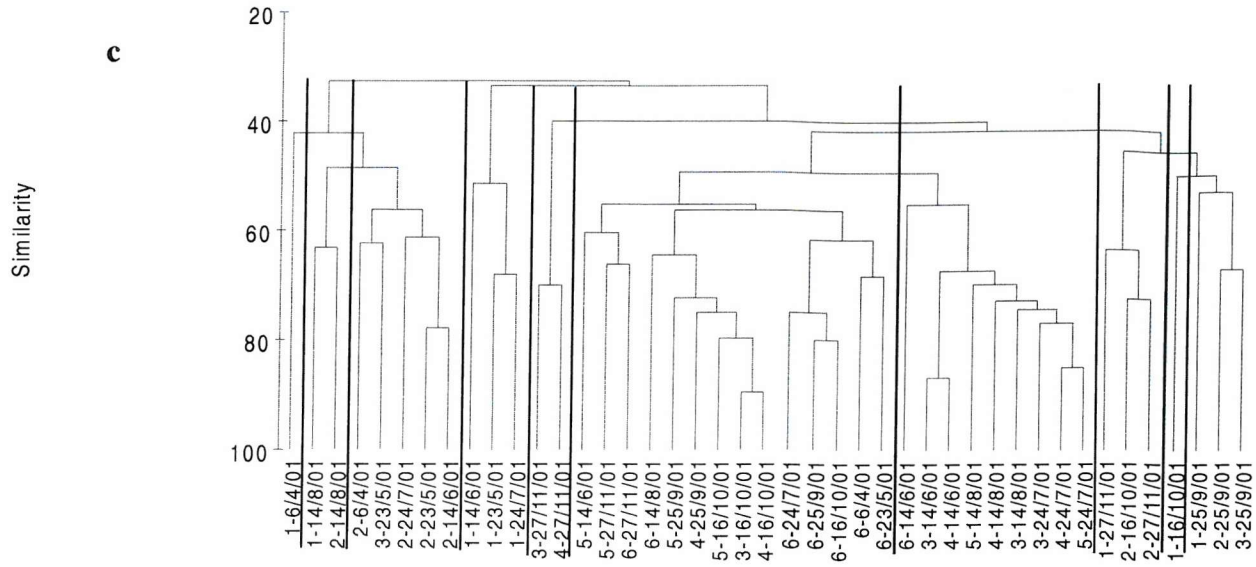


Figure 3.34: Dendogram of cluster analysis of 2001 dataset, including (a) salinity, temperature, pH, ammonium, phosphate, silicate, nitrate, N/P ratio, chlorophyll-a, rainfall and PAR data (cluster4), (b) salinity, phosphate and average PAR only (cluster5) and (c) phytoplankton abundance (cluster6). The first number of each marker refers to the station number, followed by the date.

As for the year 2000, the results indicate high dissimilarity of environmental parameters at station 1 from all other stations, however during 2001 this station was consistently dissimilar from May to November (group 2 of cluster4, Fig. 3.33a and Fig. 3.34a), while in 2000 dissimilarity of station 1 was observed from June to August. High dissimilarity of samples collected on 27/11/00 at stations 2 to 6 (group 3 of cluster4, "winter" conditions, Fig. 3.34a) was also observed. Two groups (1 and 5) were composed of only one sample, and refer to stations 1 and 2 on 6/4/01 (Fig. 3.33a and Fig. 3.34a). Group 4 of cluster4 corresponds to samples from stations 2 to 5 from May to September (samples from stations 4 and 5 were not collected in May), and reflect more homogeneous environmental conditions along stations 2 to 5 during 2001. This is in contrast to the previous year, when at least two groups were differentiated during this time at stations 2 to 4, corresponding to early and late summer conditions, mainly due to an increase in salinity and temperature at stations 2 to 4 in late July. Group 6 of cluster4 includes samples from station 6 from April to October and from stations 2 to 5 on 16/10/01. As for the year 2000, high similarity was observed between samples from stations 2 to 6 during October and November.

Using only the parameters identified on the BIOENV routine of PRIMER (salinity, phosphate and PAR) it is possible to achieve a MDS plot (MDS5) similar to the one from

the ordination of all the environmental variables measured (MDS4). This suggests that the three parameters are good descriptors of the similarity between samples collected during 2001. It is reassuring that the original groups (cluster4) can still be recognised on MDS5. For data collected during 2000 the results were not as good, and this is likely to be partially due to the larger dataset. During 2001 the sampling program was performed monthly, when the lagoon was visited 8 times in the year, in contrast to the fortnightly sampling undertaken during 2000, when 13 trips were made. The more information put into the multivariate analysis, the more difficult it is to reduce the information into few groups (of a cluster) or two dimensions of the MDS. However, it is also possible that some variables that would account for the similarity between samples in 2000 were missed during the survey.

During 2001, no marked seasonal pattern was observed from the cluster analysis, in contrast to the analysis of the 2000 dataset, and it is more difficult to follow seasonal changes from a set of monthly surveys relative to a set of bi-weekly ones.

Phytoplankton

The stress of MDS6 was 0.21 (Fig. 3.33c), which indicates a potential useful 2-dimensional picture, though any conclusions should be cross-checked against those from a cluster analysis (Clarke and Warwick, 1994). Ten species groups can be identified at approximately 45 % Bray-Curtis similarity level (Fig. 3.34c). Group 1 is composed of the sample from station 1 in April. Group 2 of stations 1 and 2 on 14/8/01, group 3 of station 2 from April to July and station 3 in May. *Cryptomonas* sp. is the dominant species in these samples, although abundances vary considerably (see Table 3.5). Group 4 includes samples from station 1 from May to July and are dominated by *C.* cf. *cohnii*. Group 5 is composed of only two samples, from stations 3 and 4 on 27/11/01. Group 6 includes samples from station 3 in October, st. 4 in September and October, st. 5 in June and from September to November and station 6 from April to November, except for the sample from June. These samples presented a diverse plankton community, including *Cryptomonas* sp., pennate diatoms and flagellates (Table 3.5). Group 7 is composed of samples from stations 3 to 5 from June to August (except for st. 5 on June) and station 6 in June. Group 8 includes samples from stations 1 and 2 in November and st. 2 in October. Group 9 is formed solely by a sample from st. 1 in October, while group 10 represents stations 1, 2 and 3 in September.

Table 3.5: Summary of main species contributing to the similarity between samples from each group of cluster6, showing average abundances in brackets. Cumulative contributions of species listed in groups 1 to 10 are respectively 100, 65, 56, 61, 56, 56, 65, 70, 65 and 64%. See appendix 12 for a graphic representation of how cluster groups are distributed with respect to sampling date and station.

Date	St.	Group	Main species (avg cells mL^{-1})
6/04/01	1	1	*Cryptomonas* sp. (425734)
	2	3	*Cryptomonas* sp. (30684)
	6	6	*Cryptomonas* sp. (75), Pennate 15 µm (12), Pennate 50 µm (5), flagellates 6 µm (19), *Strombidium* sp. 4 (4)
23/5/01	1	4	*C.* cf. *cohnii* (2379), Pennate 50 µm (11)
	2, 3	3	*Cryptomonas* sp. (30684)
	6	6	*Cryptomonas* sp. (75), Pennate 15 µm (12), Pennate 50 µm (5), flagellates 6 µm (19), *Strombidium* sp. 4 (4)
14/6/01	1	4	*C.* cf. *cohnii* (2379), Pennate 50 µm (11)
	2	3	*Cryptomonas* sp. (30684)
	3,4,6	7	flagellates 6 µm (89), *M. rubra* (89), athecate 11 µm (116), *Scrippsiella* sp. (16), *Cryptomonas* sp. (34)
	5	6	*Cryptomonas* sp. (75), Pennate 15 µm (12), Pennate 50 µm (5), flagellates 6 µm (19), *Strombidium* sp. 4 (4)
24/7/01	1	4	*C.* cf. *cohnii* (2379), Pennate 50 µm (11)
	2	3	*Cryptomonas* sp. (30684)
	3,4,5	7	flagellates 6 µm (89), *M. rubra* (89), athecate 11 µm (116), *Scrippsiella* sp. (16), *Cryptomonas* sp. (34)
	6	6	*Cryptomonas* sp. (75), Pennate 15 µm (12), Pennate 50 µm (5), flagellates 6 µm (19), *Strombidium* sp. 4 (4)
14/8/01	1,2	2	*Cryptomonas* sp. (3805), *M. rubra* (1220), *C.* cf. *cohnii* (142)
	3,4,5	7	flagellates 6 µm (89), *M. rubra* (89), athecate 11 µm (116), *Scrippsiella* sp. (16), *Cryptomonas* sp. (34)
	6	6	*Cryptomonas* sp. (75), Pennate 15 µm (12), Pennate 50 µm (5), flagellates 6 µm (19), *Strombidium* sp. 4 (4)
25/9/01	1,2,3	10	*Cryptomonas* sp. (2738), *E. marina* (596), *Cryptomonas* sp. 2 (175), *M. rubra* (227), *O. marina* (69)
	4,5,6	6	*Cryptomonas* sp. (75), Pennate 15 µm (12), Pennate 50 µm (5), flagellates 6 µm (19), *Strombidium* sp. 4 (4)
16/10/01	1	9	*C.* cf. *cohnii* (706), *Cryptomonas* sp. (251)
	2	8	*Cryptomonas* sp. 2 (572), *E. marina* (213), *M. rubra* (10)
	3,4,5, 6	6	*Cryptomonas* sp. (75), Pennate 15 µm (12), Pennate 50 µm (5), flagellates 6 µm (19), *Strombidium* sp. 4 (4)
27/11/01	1,2	8	*Cryptomonas* sp. 2 (572), *E. marina* (213), *M. rubra* (10)
	3,4	5	*Strombidium* sp. 1 (15), Pennate 50 µm (8), *M. rubra* (20)
	5,6	6	*Cryptomonas* sp. (75), Pennate 15 µm (12), Pennate 50 µm (5), flagellates 6 µm (19), *Strombidium* sp. 4 (4)

As for the year 2000, a bloom of *Cryptomonas* sp. occurred at station 1 early in the year (group 1). From May to July 2001 station 1 showed a distinct species composition from other stations, just like in June, July and August 2000. *C.* cf. *cohnii* was the dominant species during this time in 2001 at station 1 (group 4), while in 2000, *C.* cf. *cohnii* was observed from 14/6 to 8/8/00 together with *Cryptomonas* sp., *E. marina* and *M. rubra.* Cryptophytes were dominant throughout 2001 at station 2. Extremely high abundances of *Cryptomonas* sp. were observed from April to July 2001 (group 3), higher than during these months at station 2 in 2000. In both years the phytoplankton assemblage at station 1

became more similar to the one from other stations during August and September. However, cluster analysis of environmental parameters indicated high dissimilarity of station 1 from all other stations from June to August 2000 and from May to November 2001. Salinity proved to be a good descriptor of the similarity between samples from both 2000 and 2001 and it was an important factor in determining the dissimilarity of samples from station 1 in both years. Regarding the phytoplankton, the brackish end of the lagoon (Abbotsbury embayment) is spatially segregated from the rest of the Fleet from April to August (see tables 3.4 and 3.5), probably due to its high freshwater input and lower salinity. As river inflow diminishes by August and September (Fig. 3.30), more saline waters can penetrate into the embayment and conditions at station 1 become more similar to the rest of the lagoon, as happened in 2000. However, during 2001 cluster analysis of environmental data indicated that station 1 was still dissimilar in August due to lower salinities relative to the rest of the Fleet. Only in September was the salinity of station 1 more similar to the rest of the lagoon, as indicated by the grouping of sample from st. 1 on 25/9/01 in group 4 of cluster5 and of st. 1 on 14/8/01 in group 5 of cluster5 (Fig. 3.34b).

The species assemblage observed for most of 2001 at station 6 (group 6 of cluster6) was remarkably similar to the one observed for most of 2000 at this station (group 6 of cluster3).

A striking difference between the phytoplankton composition of 2000 and 2001 is the occurrence of an exceptional bloom of *P. micans* in 2000 and the total absence of this species from stations 1 and 2 in 2001. At station 3 in 2001, *P. micans* was observed in July and August at 2 and 3 cells mL^{-1}, at station 4 on the same dates its abundances were 5 and zero cells mL^{-1} while at station 5, 16 and 23 cells mL^{-1} were observed.

3.3 Discussion

Coastal lagoons in temperate zones frequently exhibit a strong seasonal gradient, both in environmental variables and plankton assemblages, because of their tight physical-biological coupling. In the Fleet lagoon, water temperature, salinity and nutrient inputs exhibited well defined seasonal patterns.

Nutrients enter the Fleet from various diffuse sources. There are seven streams which drain a relatively small catchment area of 28 km^2, much of which is under pasture with sheep and dairy farming. The contribution of each nutrient source was estimated by Mainstone and Parr (1999), and a summary is presented in table 3.6. Agriculture was shown to be the major nutrient source to the Fleet. Sewage effluents from four sewage treatment works (STW) with secondary treatment are also discharged into streams feeding

the Fleet as well as directly into the Fleet itself. Mute swans from the swannery and other bird species do not appear to make a major contribution to nutrient loads as a whole, but they may be important in the local Abbotsbury subcatchment. Other sources of nutrients considered by Mainstone and Parr (1999) were atmospheric deposition and groundwater. The latter could not be quantified due to lack of detailed information on nutrient concentrations in local groundwaters, although the authors suggest they can be potentially significant, as total inorganic nitrogen from groundwaters to the north and west of the Fleet catchment are high (around 6 mg L^{-1}). Atmospheric deposition has been shown to comprise 20 to 40 % of nitrogen inputs to estuarine and coastal waters, from industrial, agricultural and urban sources (Anderson et al. 2002), although no estimate is available for the Fleet lagoon.

Table 3.6: Summary of estimated annual nutrient loads to the Fleet, from Mainstone and Parr (1999) cited in Johnston and Gilliland (2000).

Source	Nitrogen		Phosphorus	
	Tonnes/year	%	Tonnes/year	%
Point sources (sewage works)	1.5-3.2	1-2.5	0.48-1.29	12-39
Livestock	44.4	34	1.54	37-47
Fertiliser application	64.4	49-50	0.75	18-23
Background load	18.5	14	0.42	10-13
Abbotsbury Swannery	0.3	0.2	0.06	2
Other bird species	0.2	0.1	0.06	2
TOTAL	129.3-131.0		3.31-4.12	

Figure 3.35 shows the major nutrient inputs through rivers that drain in to the Fleet. As can be seen, the Abbotsbury embayment receives the discharge of three streams, which together contribute respectively 37.4 % and 62.9 % of the annual input of nitrogen and phosphorus to the Fleet (not considering the contribution from wildfowl, estimated to represent 0.1 % for nitrogen and 2 % for phosphorus annual input). These high nutrient inputs together with the weak flushing of the west Fleet, which can be of the order of 10 days during high runoff periods and up to 40 days during drought conditions (Robinson, 1983) can lead to the accumulation of high nutrient concentrations at this site. Runoff from agricultural land is likely to result in nitrogen loading to the east Fleet. However, tidal flushing of this part of the lagoon occurs regularly and nutrient concentrations are not likely to accumulate. The Fleet at Abbotsbury (station 1) has by far the largest nutrient concentrations, which decreases eastwards down the lagoon. The Narrows and Smallmouth (stations 6 and 7) showed the lowest concentrations of all nutrients.

The inputs of nitrate, ammonium and silicate and that of phosphate showed an alternating seasonal cycle. This is consistent with the findings of Mainstone and Parr

(1999), who reported a strong annual cycle in nitrogen inputs to the Fleet, with winter highs and summer lows and that most of the nitrogen inputs enter the lagoon through streams, from agricultural sources (Table 3.6 and Fig. 3.35). In the present study, nitrate concentration was extremely high in autumn, in mid-October and early November 2000 and mid-October 2001, although no measurements were made over winter months of December – March. The increased nitrate concentrations during the wet period (see Fig. 3.27), at the end of autumn towards the winter and in April 2001, suggest an input through freshwater, by either direct runoff or via streams.

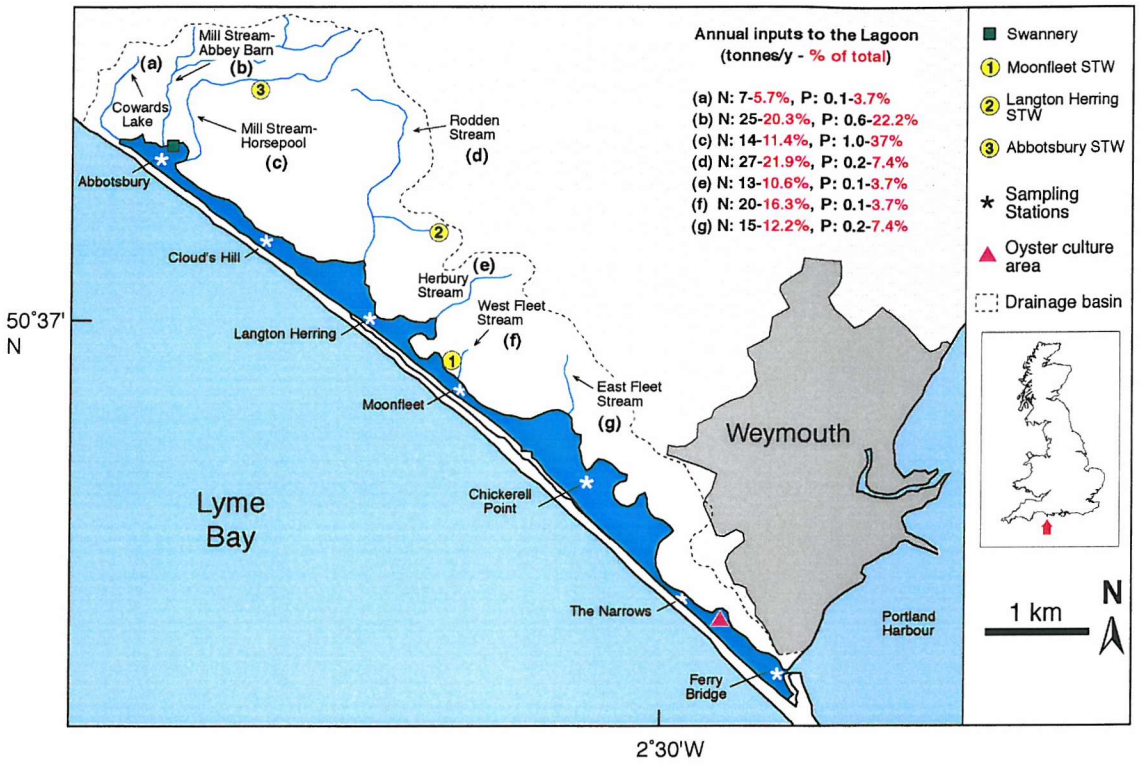


Figure 3.35: Map of the Fleet lagoon showing main annual nitrogen and phosphorus inputs.

From May to September in both years nitrate was lower than 1 μM at stations 2 to 7 and these low values are probably linked to eelgrass and seaweed assimilation. However, at station 1 higher concentrations were observed, especially during 2001, even during the summer. In 2000, the high concentration of nitrate detected at station 1 was exhausted only after two phytoplankton blooms, in mid June following growth of cryptophytes, and in September, after a *P. micans* bloom.

Apparently, sewage does not significantly contribute to the nitrogen input to the Fleet, although it is a considerable phosphorus source (Mainstone & Parr, 1999 - Table 3.6). Ammonium concentrations remained low even at station 1 probably due to intense phytoplankton and seaweed and eelgrass assimilation. A peak in July 2000 may have been

caused by the input from birds, particularly swans faeces. There are a number of species of waterfowl that inhabit the Fleet, and the mute swans tend to congregate at the Abbotsbury embayment during most of the year, associated with the Swannery. High ammonium concentrations were observed in October and November in both years and it is likely that rainfall and freshwater runoff from adjacent agricultural land are also significant ammonium contributors to the system.

Silicate concentrations were very high during most of the year, decreasing during the summer. High values were observed at stations 2 to 6 from September 2000 and were associated with the larger freshwater input that flushed the whole lagoon during this very wet period. In 2001, silicate concentrations near to or higher than 100 μM were observed at station 1 in April-May and October-November, periods that followed increased rainfall, but silicate at other stations was not as high as the previous year during autumn.

It is estimated that 50 to 70 % of the annual total phosphorus input to the Fleet comes from agricultural sources (livestock + fertiliser) (Table 3.6). Contributions from two sewage works, at Abbotsbury and Langton Herring are less important, but may contribute 12 to 40 % of the annual phosphorus input to the Fleet (Table 3.6). This nutrient showed an alternating seasonal cycle, peaking during the summer in both years, in contrast with ammonium, nitrate and silicate. The discharge of sewage effluents from Abbotsbury STW into Mill Stream Horsepool (Fig. 3.35) during the time of lowest river flow and consequently poor effluent dilution, together with the input from wildfowl, particularly swans, into this region of weak flushing must have contributed to the build up of high phosphate concentrations at station 1 observed in summer during both years. However, during summer 2001 phosphate was higher not only in the Abbotsbury embayment (st. 1) and Langton Herring (st. 3), that receive sewage discharge, but increased phosphate values were observed throughout the lagoon (Fig. 3.10b). This suggests an internal source of phosphate, probably released from organic rich sediments of the lagoon, as observed in Mediterranean coastal lagoons, where phosphate is released from the sediments during high temperature periods (in summer) when rates of decomposition of organic matter in the sediment are maximal (Nixon, 1982, Moutin et al. 1993).

A similar seasonal pattern of alternating nitrate from runoff sources during winter and phosphate inputs from sewage discharge and sediment release during summer has been observed in other temperate coastal lagoons, like the Mar Menor, on the southeastern coast of Spain (Gilabert, 2001), the Thau lagoon, in southern France, in three coastal lagoons of Amvrakikos gulf, Greece (Kormas et al. 2001) and in the lagoons studied by Nixon (1982).

Johnston & Gilliland (2000) observed a double annual cycle in phosphorus loads to the Fleet, with winter and summer highs. The winter highs were related to stream inputs, particularly Mill Stream Horsepool and Mill Stream Abbey Barn (Fig. 3.35), while summer highs were related by these authors to wildfowl inputs. According to Johnston & Gilliland (2000), the impact of the wildfowl may be exacerbated owing to associated high organic loadings, resulting in increased anoxia within sediments and enhanced phosphate release.

Considering that birds were important contributors to the phosphate inputs to the Abbotsbury embayment during the summer, it would have been expected that ammonium concentrations should also have increased during this season. This assumption is based on the fact that birds are uricotelic animals, which means that they excrete nitrogen as uric acid. However, significant amounts of other nitrogenous compounds, particularly ammonium, but also urea, creatine and amino acids, have been reported in the urine of domestic fowl and ducks (Shoemaker, 1972). Nevertheless, ammonium concentrations remained low throughout the summer period at Abbotsbury.

A peak in ammonium concentrations was observed in July 2000 at station 1, at the same time that phosphate started to increase, but ammonium concentrations decreased very rapidly, probably due to rapid consumption by phytoplankton and macroalgae. It is possible that the ammonium provided by bird faeces throughout the summer is quickly assimilated by plants and did not accumulate in the water. In contrast, phosphate concentrations gradually increased and peaked in late summer, decreasing in early September 2000, when chlorophyll-a was 69.5 μM at station 1. The high phytoplankton biomass observed during late summer 2000 in spite of low ambient nitrogen concentrations measured suggests a tight coupling between nitrogen input to the system and phytoplankton assimilation. This could be an indicator that nitrogen is a possible limiting factor for phytoplankton growth during summer, as the supply of ammonium was rapidly consumed by phytoplankton, while high phosphate concentrations accumulated in the water. Indeed, nitrogen is often the nutrient that first limits primary production in shallow coastal lagoons and at the estuarine interface, between marine and freshwater habitats (Fong et al. 1993, Anderson et al. 2002).

Reynolds (1997) compared a selection of reported species-specific maximal phosphate uptake rates and their half-saturation concentrations and came to the conclusion that the concentrations that half-saturate these rates fall substantially below 0.1 μM, and that the growth rate-saturating phosphate requirement is an order of magnitude lower. According to this author it can be safely deduced that phosphorus is not limiting growth

for as long as the concentration of soluble reactive phosphate (SRP) is not less than 0.25 μM, and often rather less than 0.1 μM. So phosphorus can not be assumed to be limiting as long as there is 0.1 to 0.2 μM in the water. On the basis of rather fewer original data sets and using the same approach, Reynolds (1997) estimated that a concentration of dissolved inorganic combined nitrogen (nitrate + nitrite + ammonium) in excess of 7 μM was sufficient to saturate the needs of phytoplankton growth.

Considering the values of 0.25 μM of phosphorus and 7 μM of total inorganic nitrogen as threshold between non-limiting and limiting conditions, it can be seen that nutrient conditions are not likely to be limiting phytoplankton growth at the Abbotsbury embayment, except for periods following intensive growth (bloom conditions). At stations 2 to 7 nitrogen limitation is likely to take place from spring to autumn, and phosphorus is not likely to be limiting.

The relative abundance of nitrogen to phosphorus (N:P ratio) has been shown to affect phytoplankton composition. In Tolo Harbour in Hong Kong, it was shown that the number of dinoflagellate red tides increased as the annually averaged N:P ratio fell from 20:1 to 11:1 between 1982 and 1989 (Anderson et al. 2002). Hodgkiss (2001) cited in Anderson et al. (2002) showed that whenever the N:P ratio fell below around 10:1 in Tolo Harbour, dinoflagellate cell numbers increased. These correlations were consistent with experimental data, that showed that *P. micans*, one of the major dinoflagellate species blooming there, has optimal N:P ratios for growth of 5-10. In the Fleet lagoon, N:P ratios were indeed lower than 11:1 from June to September in both years, and was 4:1 in early July 2000 at station 2, when *P. micans* started to bloom, decreasing even further later on, as nitrogen was preferentially consumed. N:P ratios lower than the Redfield 16:1 found in the ocean are usually found in lagoons and in other coastal marine waters (Nixon, 1982). Measurements of inorganic nutrient fluxes across the sediment-water interface in a variety of nearshore marine environments have shown that the N:P ratio of this flux is much lower than the 16:1 usually found during pelagic remineralization. This effect is compounded by lower freshwater input during summer and by the larger biological uptake of nitrogen relative to phosphorus (Nixon, 1982). Exceptions to this general trend are periods when there is a large input of freshwater (rich in nitrogen).

The importance of the dissolved organic nitrogen pool, urea in particular, has been demonstrated for blooms of *Prorocentrum minimum* in estuarine fish ponds (Anderson et al. 2002). Urea now comprises roughly 40 % of all nitrogen fertilizers produced in the world (Constant and Sheldrick, 1992 cited in Anderson et al. 2002), indicating that its proportion in runoff of agricultural land can be quite high. This potential increased input

of urea to the Fleet lagoon, where runoff is a significant source of nitrogen, could have important implications for phytoplankton species composition in this lagoon. However, dissolved organic nitrogen concentrations in the Fleet are not known.

Nutrient inputs to the weakly flushed west Fleet have raised concern about eutrophication of the lagoon. John (1995) reported similar nitrate and ammonium concentrations in summer 1995 in the Fleet lagoon, however, phosphate concentrations reported here during summer are about twice as high as those observed by John (1995) for the same period in 1995. The Environment Agency (EA, 1998) sampled the Fleet lagoon in April and July 1996 and March, July and August 1997. These samples were taken during two days at low and high tide at neap and spring tides, in the mid channel from a boat close to the stations 1, 3, 5, 6 and 7 used in this study. Comparing nutrient values reported by the EA with the ones described here for the same months, it can be seen that phosphate and ammonium values reported here were much higher than those reported by the EA, while nitrate and silicate were lower. This difference could indicate a recent increased supply of ammonium and phosphate to the Fleet, but could also be a sampling artefact. Sampling in this study was carried out from the shore, while it was from the mid channel by the EA.

Shallow coastal lagoons are particularly susceptible to nutrient enrichment because of limited tidal and freshwater flushing. General conceptual models have been proposed that show typical lagoon response to nutrient enrichment as a sequential change in the dominant primary producers (Nienhuis 1992, Castel et al. 1996, Raffaelli et al. 1998 cited in Eyre and Ferguson, 2002). According to these models, benthic primary producers such as seagrasses and benthic microalgae decrease, epiphytes build up and are in turn replaced by pelagic primary producers such as phytoplankton and fast-growing floating macroalgae as nutrient enrichment progresses.

Seaweeds and eelgrass are important primary producers in the Fleet lagoon, where a diverse community has been reported to occur (Holmes, 1993). There is a diverse assemblage of macroalgae at the marine end of the lagoon that is replaced at Chickerell (st. 5) by *Zostera* spp. beds (eelgrass) and further upstream by *Ruppia cirrhosa*, *Lamprothamnium papulosum*, *Zostera noltii* and *Ruppia maritima* until Cloud's Hill (st. 2) (Dyrynda, 1997). Moreover, it seems that the upper and lower limits of distribution of *Zostera* and *Ruppia* did not change between 1968 and 1991 (Holmes, 1983 and 1993). At Abbotsbury, a number of filamentous species of mainly *Cladophora* and *Enteromorpha* occur, *Chaetomorpha linum* is commom, and in summer can be accompanied by *Ulva lactuca*.

However, epiphytic growth on all seaweeds and *Zostera* was reported to be well developed since the late 1960's and probably earlier (Johnston and Gilliland, 2000). It is not known if this was an early sign of the effects of nutrient enrichment upon the macroalgae community, or if it was always common. At Abbotsbury, *Zostera* has been reported to be rare at least since 1978 (Whittaker, 1980) and *Ulva lactuca* was present at least since 1981 (Burrows, 1981), giving some indication that conditions in this region were not pristine by that time. Burrows (1981) reported that there was a good deal of natural pollution in the form of organic matter from rotting vegetation and from bird droppings at the western end of the Fleet and that this could be stimulating the growth of *Ulva lactuca*. Phytoplankton blooms have been recorded to occur at Abbotsbury on a number of occasions since 1969 (Whittaker, 1980, Saunders-Davies 1993, John, 1995, EA 1998), particularly during warm and dry summers, suggesting the importance of water exchange at Abbotsbury in controlling these events. In 1969 and 1976 blooms were thought to be triggered by farm pollution and there was associated fish mortality, but the phytoplankton species involved were not identified.

Data presented here suggests that phytoplankton are significant primary producers from April to November only in the west Fleet, particularly at Abbotsbury, in spring and late summer. In the mid and east Fleet, low chlorophyll-a values during summer are an indication that macroalgae and eelgrass are outcompeting phytoplankton during this period. Indeed, Fong et al. (1993) demonstrated that, under nutrient limiting conditions, macroalgae were important in controlling the abundance of phytoplankton, and that macroalgae were superior competitors for nitrogen. The formation of allelopathic compounds by macroalgae and competition for micronutrients have already been cited to explain low levels of phytoplankton during summer in the presence of macroalgae (Fong and Zedler, 1993). Moreover, the interaction between attached algae and phytoplankton is dependent on the amount of benthic substrate compared to water volume, and the ratio of surface area to volume in shallow lagoons is high, benefiting the attached forms (Fong et al. 1993). In the Fleet it is also likely that drifting floating masses of filamentous green macroalgae create shading for phytoplankton growth during summer in the mid and east Fleet. Chlorophyll-a can be high in this region by late summer and autumn (when macroalgae and eelgrass have died off) when the increasing freshwater input flushes the lagoon transporting the high chlorophyll-a waters of Abbotsbury eastwards, as in 2000; but it is unlikely that *in situ* growth producing high phytoplankton abundances will occur in the mid and east Fleet.

Linkages between more frequent and intensive harmful algal blooms and eutrophication have been noted within the past two decades in a number of coastal waters (Paerl, 1988, Richardson, 1997, Anderson et al. 2002). As eutrophication progresses, shifts in phytoplankton communities towards declines in certain diatom species in favour of nanoplankton and flagellates are expected. A more frequent response to nutrient enrichment occurs when a species or group of species begins to dominate under the altered nutrient regime (Anderson et al. 2002). The mechanisms of bloom species selection, and the causes of the shifts in phytoplankton community structure favouring flagellate taxa and their blooms are major unresolved harmful algal bloom issues (Smayda and Reynolds, 2001).

Indeed, flagellates (including dinoflagellates) represented from 53 to 93 % of total phytoplankton abundances and 40 to 97 % of total phytoplankton carbon content in the Fleet lagoon during the two years studied, lower values being associated with the marine end of the lagoon. Diatoms, on the other hand, were responsible for between 5.5 and 34 % of total abundances and 1 to 43 % of total carbon, with the higher values only observed at the mouth of the lagoon (Table 3.7).

Table 3.7: Mean percent contributions of flagellates and diatoms to total cell abundance and total carbon content at each station in the Fleet lagoon during 2000 and 2001.

Year	2000				2001			
	Cell abundances		Carbon content		Cell abundances		Carbon content	
Stations	Flagellates	Diatoms	Flagellates	Diatoms	Flagellates	Diatoms	Flagellates	Diatoms
1	93	5.5	97	2	81	13	90	4
2	86	11	85	11	90	1	84	1
3	78	17	85	10	63	15	63	6
4	86	7	95	1	55	10	51	4
5	75	15	85	5	67	14	45	19
6	81	13	75	11	53	34	40	35
7	61	34	44	43	-	-	-	-

In the present study, blooms of cryptophytes occurred in spring 2000 and 2001 at Abbotsbury and Cloud's Hill (particularly in 2001) and exceptional chlorophyll-a concentrations of 163 $\mu g\ L^{-1}$ in June 2000 and 290 $\mu g\ L^{-1}$ in April 2001 were recorded. Dense "green" algal blooms were previously reported to occur at Langton Herring (st. 3) during April/May 1995 and 1996 by Dyrynda (1997). Although chl-a was not measured and the species was not identified in Dyrynda's work, the time of occurrence suggests that these blooms were of cryptophytes. Nutrient conditions measured in early April 2001 indicate that there was plenty of nitrogen and phosphorus by the time the cryptophytes bloom started, due to winter input through rainfall and runoff. Cryptophytes are able to

self-replicate more rapidly due to their small dimensions, high surface area to volume ratio, which favours rapid surface exchange (Reynolds, 1997). In this way they were able to take advantage of the rich nutrient conditions. Cryptophytes have the ability to acclimate to light climates that would be limiting for other phytoplankton species by raising their chlorophyll-a content, maximizing cell specific potential for light harvesting under low light conditions (Reynolds, 1997). Cryptophytes can form blooms even during the winter in the Mediterranean hypersaline Mar Menor coastal lagoon (Gilabert, 2001).

Scrippsiella sp. was observed in both years between June and August at stations 1 to 5. Higher numbers were observed at station 2, particularly in August and early September 2000. The existence of cyst beds of *Scrippsiella rotunda* along the Fleet suggests that this is the species found in the plankton, although identification to the species level has not been done. It is likely that germination of cysts plays an important role on the species population dynamics. Resting cysts of dinoflagellates are known to have a mandatory dormancy stage before excystment, ranging from 6 weeks to 5 months (Guillard and Keller, 1984). A decrease in cyst abundances was observed between 14/6/01 and 24/7/01 at station 2, suggesting germination of *S. rotunda* cysts from the sediment after stimulation by appropriate environmental signals; and resulting in the *Scrippsiella* population observed in the water column on 24/7/01. Cyst abundances increased considerably on 25/9/01, suggesting that *Scrippsiella* cells have encysted and settled on to bottom sediments by this date. This is supported by the fact that *Scrippsiella* was not found in the water column later in the year. Although there are no studies about cyst germination of *S. rotunda*, the findings of Binder and Anderson (1987) from their laboratory study of *S. trochoidea* suggest that with the short dormancy period of the cyst, the fairly broad temperature range for germination and growth, rapid cycling of the *S. trochoidea* population between motile and resting stages is possible.

A intense bloom of the dinoflagellate *P. micans* was observed in the west Fleet in late summer-autumn 2000. It was initiated at station 2 in July 2000, resulting in a chlorophyll-a peak value of 244 $\mu g\ L^{-1}$ in September at Abbotsbury. This bloom was not observed in 2001. It has been demonstrated that seasonal phytoplankton blooms can be triggered by seasonal fluctuations in river flow. In some shallow coastal ecosystems blooms can develop only when the river discharge falls to a level at which the water residence time is longer than the phytoplankton population doubling time (Cloern, 1996). In north San Francisco bay, it was shown that most of the interannual variability in chlorophyll-a concentrations for the period from 1974 to 1995 was driven by two

hydrodynamic processes, one associated with fluctuations in river flow, and one associated with managed diversions of freshwater from the upper estuary (Cloern, 1996).

The river Wey gauge data showed that in general mean river discharge is higher from January to March, decreasing gradually throughout the spring and reaching lowest values from July to October, after when it increases again. When comparing Wey river flow during 2000 and 2001, it can be seen that river flow was extremely high from November 2000 to May 2001, during the wettest winter on record. Indeed, November and December 2000 and April 2001 were the highest mean flows ever recorded in these months.

Although there are no gauge data for rivers that drain to the Fleet lagoon, the Wey river data should give an indication of how the flow of these rivers varied during the period of this study. Variations in the freshwater input to the Fleet will have a direct effect on salinity distribution along the lagoon, and particularly in the Abbotsbury embayment, that receives the major freshwater input to the Fleet. As stated by Robinson (1983), the salinity regime is apparently controlled by the balance between freshwater runoff and tidal flushing, leading to longitudinal salinity profiles which fluctuate over a tidal cycle, over a fortnightly spring neap cycle, and with the variation of precipitation.

Salinity at stations 1 and 2 were consistently lower during the whole of 2001 relative to 2000. This implies that due to the increased freshwater input to the west Fleet in 2001, the saline waters that enter the lagoon in the east Fleet did not penetrate as far in the lagoon during 2001 relative to 2000. Moreover, the cluster analysis based on environmental data showed high dissimilarity of station 1 in relation to the rest of the lagoon from June to August 2000 and from May to November 2001. The dissimilarity through a longer period in 2001 was a result of lower salinities at Abbotsbury during the whole year.

In 2000, *P. micans* was initially observed in significant numbers at station 2 by 11/7/00 (68 cells mL^{-1}), when salinity was 28.1. By 5/9/00 cell numbers had increased to 25661 cells mL^{-1}. Salinity at station 2 during this period varied between 28.1 and 31.8. The *P. micans* net increase rate during this period, here used in the context of accumulation of *P. micans* cells, and calculated as $\ln (N_t/N_0)/t$ was remarkably constant and varied between 0.11 and 0.13 divisions per day, except for the period between 25/7 and 8/8/00, when it was 0.06 div day^{-1}, coinciding with a drop in salinity, that might indicate flushing of cells. It is likely that *P. micans* cells observed in the lagoon originate from coastal waters and that these cells enter the Fleet during flood tides. Once inside the lagoon, *P. micans* meets favorable nutrient conditions for growth and a bloom can

develop, however, cell losses can also occur during ebb tides or after increased freshwater inputs that can flush cells eastwards in the Fleet. If the freshwater input to Abbotsbury is low, water residence time at this end can be quite high, up to 40 days, and cells will be retained under favorable growth conditions, and will be able to reach high biomass, as growth surpasses losses due to flushing, sedimentation and grazing pressure.

At station 1, low *P. micans* numbers were recorded on 25/7/00 (17 cells mL^{-1}), when salinity was 24.2, and on 8/8/00 (25 cells mL^{-1}), at salinity of 21.4. Bloom numbers were only observed at station 1 from 22/8/00 (949 cells mL^{-1}), when salinity had increased to 26.2, indicating advection of cells from station 2 by the flood tide.

In 2001 a different scenario took place. Due to increased freshwater input, salinity at station 1 was consistently lower from April, was 17.6 by 14/8/01, varied between 21.3 and 30.8 from 3 to 25/9/01, when it was 28.7. Lower salinities were also observed at station 2, for example, 26.4 on 24/7/01 contrasting to 32.3 on 25/7/00, and salinity reached a value of 30 at station 2 only in late September 2001. However, it is acknowledged that a continuous salinity monitoring dataset would be more appropriate than discrete samples to verify these interannual variations in salinity values at the west Fleet. According to Robinson et al. (1983), there is a marked attenuation of tidal amplitude at the west Fleet, where tides have a strong fortnightly component, although the semidiurnal tide propagates only weakly when the mean level is high at spring tides, and not at all when the mean level is low at neaps.

It seems that during 2000, marine waters moved further into the lagoon than in 2001, due to reduced freshwater inputs, thus transporting *P. micans* cells to the area where suitable growth conditions provided high nutrient stocks (station 1 and 2) during late July-August, when peak water temperatures were recorded and seaweeds and eelgrass were dieing off. The combined effect of these favorable conditions for growth and longer water retention times, probably determined the development of the *P. micans* bloom in 2000. The confinement of the water and the minimal loss through advection created suitable conditions for *P. micans* to develop high abundances over a period of several weeks in the western Fleet in 2000. As stated by Steidinger (1983) and Paerl (1988) a bloom is not caused by sudden population explosions due to increased division rates, but instead represents normal population increases that are confined (growth rate exceeds division and loss) or physically concentrated by boundary layers, frontal systems or convection cells, sometimes in concert with the organism's vertical migratory behaviour. Therefore, in the Fleet the horizontal boundary between freshwater and marine coastal waters physically concentrated *P. micans* cells in a favourable nutrient rich region.

In contrast, during 2001, due to the increased freshwater input to the west Fleet, marine waters did not penetrate so far into the lagoon. This probably determined that *P. micans* did not reach the nutrient rich conditions of stations 1 and 2 at all during 2001. When the more saline waters reached station 2 in late September 2001, *P. micans* was no longer observed in the water column. This suggests that annual recruitment of *P. micans* into the Fleet lagoon requires seeding from coastal waters. Therefore, the size of the coastal *P. micans* inoculum may partially explain interannual variations in *P. micans* bloom occurrence and magnitude in the Fleet.

A bloom of *P. micans* that discolored the waters of the Fleet was reported to occur for more than 4 weeks at the Abbotsbury embayment in August 1997 (EA, 1998). Wey river flow data shows that 1997 was a year of relatively low river flow from January to October (generally lower than the historic mean), suggesting that marine salinities were probably able to penetrate into the lagoon up to Abbotsbury, transporting *P. micans* cells to this region.

Other authors (Tyler and Seliger, 1978, Ohman and Lindholm, 1995) have described blooms of *Prorocentrum* in bays and estuaries after inoculation from coastal waters. It seems that once *Prorocentrum* is transported to enclosed, nutrient rich waters, favourable growth conditions are met and blooms can develop.

The optimum salinity for growth of *P. micans* was found by Kain and Fogg (1960) to be 25. However, Cannon (1996) did not find significant differences in the growth rate of *P. micans* grown at salinities of 21, 26, 31.5 and 35, although cells grew faster at a salinity of 26. This data suggests that *P. micans* is able to maintain high growth rates over quite a wide range of salinities, as long as suitable nutrient and temperature growth conditions are met. In Poulnaclogh bay in Ireland, high abundances of *P. micans* were observed in an area of low flushing time, reaching chlorophyll-a values of 185 $\mu g\ L^{-1}$ (Pybus, 1990). Moreover, it has been demonstrated that *P. micans* is able to use a migratory response as a method for the avoidance of flushing from a pond (Filipic & Revelante, 1985).

Other factors may play a role in determining *P. micans* blooms in the Fleet, such as lack of predation, although there is no study about the zooplankton of the lagoon, and the magnitude of cell loss due to grazing by microzooplankton in the Fleet is not known. However, increased concentrations of phaeopigments from 5/9/00 to 3/10/00 (particularly for the latter date, see Fig. 4.3) at station 1 suggest the senescence of the *P. micans* bloom and/or an increased grazing pressure upon this species (see chapter 4). Another factor that might contribute to *P. micans* bloom formation in the west Fleet is the ability to use

HCO_3^- indirectly through the catalytic production of CO_2 by extracellular carbonic anhydrase activity under conditions of CO_2 limitation, i.e. high pH values, as shown in cultures by Nimer et al. (1999). At high pH levels, the availability of CO_2 decreases and may become limiting to photosynthesis and growth of some phytoplankton species (Chen and Durbin, 1994). Moreover, *P. micans* has been reported to be a mixotrophic species (Jacobson and Anderson, 1996), and the ability to switch between modes of nutrition represents a significant survival mechanism.

Other dinoflagellate species were observed together with *P. micans*, including *Scrippsiella* sp. and some athecate dinoflagellates. This same species assemblage has also been observed in the shallow coastal Ganzirri lagoon, in Italy (Giacobbe et al. 1996). *Scrippsiella* sp. and athecate dinoflagellates were present in the west Fleet during 2001, in the absence of *P. micans*. *Scrippsiella* sp. has probably an autochthonous origin from cyst beds inside the lagoon, in contrast to *P. micans*, that probably originates from coastal waters. Therefore, these two species present different recruitment strategies that will provide an inoculum or seed population that is a prerequisite for a dinoflagellate bloom.

The mechanisms behind the *P. micans* bloom in the west Fleet lagoon are summarized in Fig. 3.36. The Wey river discharge gives an indication of the variability of the freshwater input to the Fleet lagoon during 2000 and 2001. Wey river flow data suggests that the lagoon received a massive input of freshwater during the period between November 2000 and May 2001, in contrast to the period between January and May 2000 (Fig. 3.36a). The increased freshwater input to the Fleet during 2001 caused a different salinity structure in the lagoon, and lower salinity values were measured at Abbotsbury (st. 1) in 2001 relative to 2000 (Fig. 3.36b, c). Lower salinity in the west Fleet suggests that the marine waters are not penetrating as far into the lagoon as during the previous year, a crucial factor for the advection of *P. micans* cells to the west Fleet. Therefore, although nutrient, light and temperature conditions in the west Fleet were similar between the two years studied, the *P. micans* inoculum that originates from coastal waters outside the lagoon did not reach the innermost part of the Fleet in 2001 (Fig. 3.36e). In contrast, during 2000, *P. micans* cells were advected to the west Fleet, reaching the area of suitable growth conditions of reduced turbulence, high water residence time and high nutrient concentrations, attaining exceptionally high abundances (Fig. 3.36d). As a comparison, *Scrippsiella* sp., a dinoflagellate that presents a different strategy and originates from cyst beds that are more abundant at station 2, germinated from bottom sediments during July in both years, reaching similar numbers at station 1 in 2000 and 2001 (Fig. 3.30f, g). At station 2, higher numbers of *Scrippsiella* sp. were found in 2000 relative to 2001, probably

as a result of the confinement of these cells in this region caused by the balance between freshwater and saline water, the same process that determined the development of *P. micans* bloom abundances. Studies relating dinoflagellate cysts inoculum size and the magnitude of a bloom in the natural environment suggest that hydrological and meteorological conditions regulate the bloom dynamics once the vegetative population is established (Kremp, 2000 and references therein).

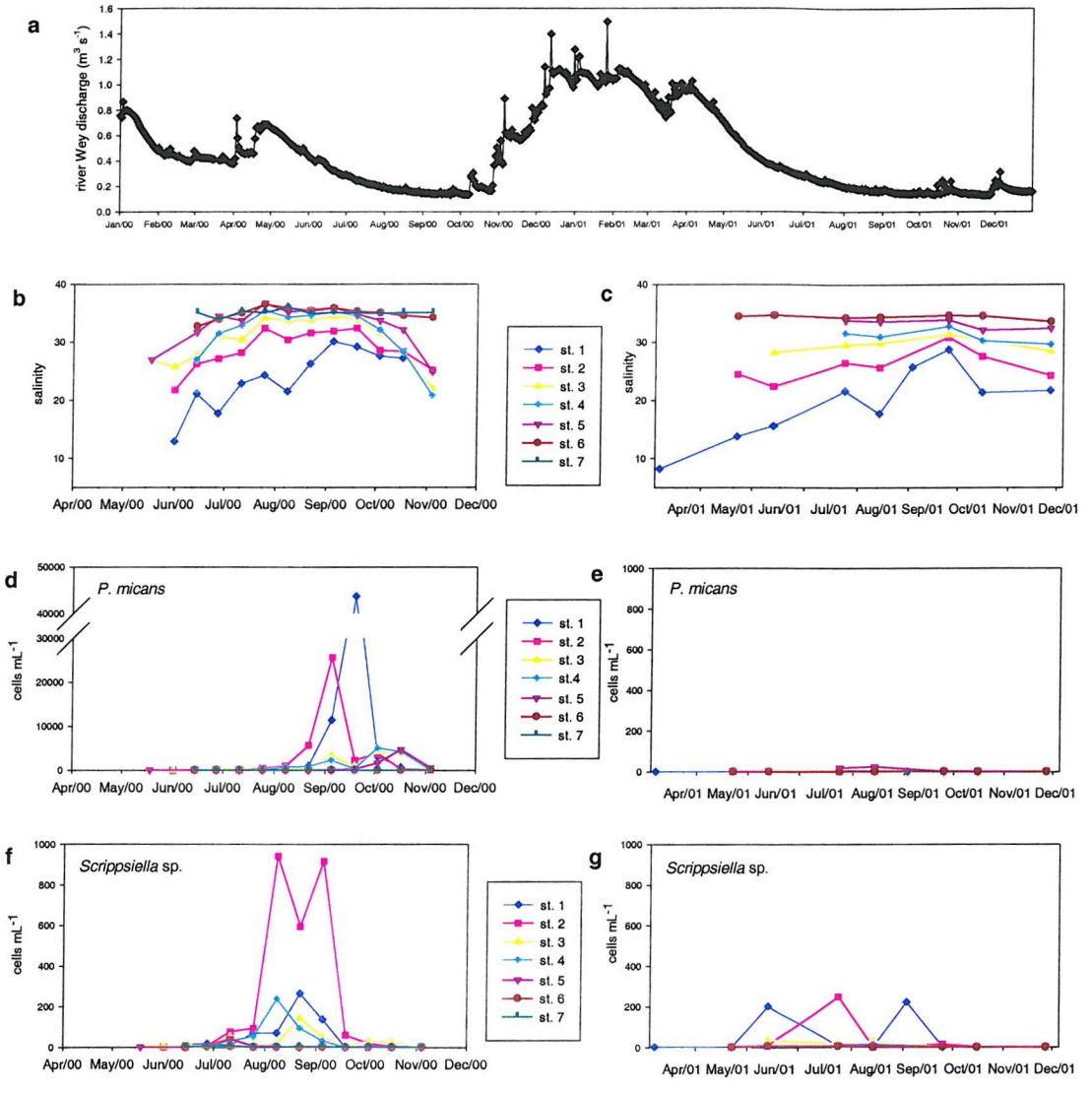


Figure 3.36: A summary of factors contributing to the interannual variability observed between *P. micans* abundances at stations 1 and 2 in 2000 and 2001, in contrast to *Scrippsiella* sp. River Wey discharge (m^3 s^{-1}) (a), salinity during 2000 (b) and 2001 (c), *P. micans* abundances during 2000 (d) and 2001 (e), *Scrippsiella sp.* abundances during 2000 (f) and 2001 (g).

Smayda and Reynolds (2001) grouped HAB dinoflagellate species into nine type groups according to life-form properties based on morphotype (size and shape) and habitat preferences, taking the approach that life form properties take precedence over

phylogenetic properties in the selection of species responsible for a particular HAB event. They were able to distinguish nine types of HAB bloom events reflecting an array of HAB species with distinct morphotype features and specific habitat preferences along an onshore-offshore mixing-nutrient gradient. Nutrient rich lagoons can be incorporated into their approach, and are home of "C-strategists" euglenophytes (like *E. marina*) and to small gymnodinoids (like the athecate species), peridinians (like *Scrippsiella* sp.) and prorocentroids (like *P. micans*). "C species" are primarily invasive species which often predominate following onset of elevated nutrient conditions and are generally small, have faster growth rates and achieve greater abundances. It is noteworthy how successfully this classification scheme can be applied to the phytoplankton of the Fleet lagoon, indicating common physiological strategies of the dinoflagellate species that occur together in the lagoon, as highlighted by the "Life form Intaglio" developed by Reynolds for freshwater phytoplankton species and applied by Smayda and Reynolds (2001) to marine dinoflagellates.

Diatoms were an important component of the phytoplankton of the Fleet only at the marine end of the lagoon and during some short periods during spring at stations 2 and 3. It is possible that diatoms are more abundant during the winter and early spring at these stations, but unfortunately we have no data for this time of the year. Many authors have argued that non-diatoms have been stimulated by following changes in the availability of nitrogen or phosphorus relative to silicate. This assumption is based on the fact that silicate is not abundant in sewage effluent, but nitrogen and phosphorus are, and that the N:Si or P:Si ratios in some estuaries and coastal waters have increased over recent decades, favouring non-diatom species. However, this argument does not seem to apply for the Fleet, as considerable silicate inputs enter the lagoon through runoff, and silicate concentrations can be quite high, particularly in the west Fleet. Indeed, N:Si and P:Si ratios were quite low during both years, being less than 1 during most of the time.

Diatoms have been highly correlated with large and/or frequent additions of nitrate, in part because they have physiological adaptations which allow them to exploit nitrate-rich conditions (Rios et al. 1995, Lomas and Glibert, 1999, Anderson et al. 2002). Microflagellates, including dinoflagellates, are more frequently associated with low nitrate concentrations, higher ammonium, urea, or DON supply, and consistently show physiological preference for reduced forms of nitrogen (Rios et al. 1995, Lomas and Glibert, 1999, Anderson et al. 2002). In the Fleet, apart from at Abbotsbury, where high nitrate and ammonium concentrations were measured, ammonium concentrations were higher than nitrate during spring and summer, although concentrations of both nutrients

were quite low. This may be another factor determining the success of flagellates in the Fleet.

A number of heterotrophic species flourished at station 1 and 2 throughout 2001. The most abundant, *Crypthecodinium* cf. *cohnii*, has been reported to occur in nature usually among decaying seaweeds. Some strains of *Crypthecodinium* have been reported to grow axenically in organic medium (osmotrophically), and some shown to prey on cells of the red microalgae *Porphyridium* sp. myzocytotically, i.e. the cell content of the prey is ingested by a feeding tube, leaving the prey's empty cell coat in the medium (Ucko et al. 1997). *Oxyrrhis marina* is a heterotrophic dinoflagellate that feeds on cryptophytes (*Rhodomonas* sp.) and *Gyrodinium instriatum* has been reported to grow mixotrophically (Jacobson and Anderson, 1996). The ciliate *M. rubra* ingest cryptophytes and retain their organelles, a phenomena that was shown to be necessary for the sustained rapid growth of an isolate in culture (Gustafson et al. 2000).

For the heterotrophic dinoflagellates *Gymnodinium sanguineum*, *Gyrodinium uncatenum* and *Ceratium furca*, that prey on < 20 μm ciliates like *Strombilidium* and *Strombidium* in Chesapeake bay (Bockstahler and Coats, 1993), feeding showed a significant positive correlation with prey abundance. *Gyrodinium galatheanum* is another heterotrophic species from Chesapeake bay that is known to graze on cryptophytes and several other nanoplanktonic organisms, and the number of prey ingested was positively associated with cryptophyte abundance (Li et al. 2000). Gustafson et al. (2000) also state that the presence of cryptophytes prey may trigger *M. rubra* blooms. It is likely that the constant availability of cryptophyte prey during 2001 favoured the development of a higher heterotrophic biomass. The constant and high availability of *Cryptomonas* sp. in the Fleet associated with high numbers of *Crypthecodinium* may suggest that this species is feeding on this organism as well, although this has not been reported before. The co-occurrence of certain heterotrophic species with high concentrations of a given type of prey species may imply food preferences (Hansen, 1991).

Nutrient enrichment of the western end of the Fleet increases the probability of blooms of cryptophytes and dinoflagellates, but in itself, probably does not trigger their development. Other factors like the balance between freshwater and saline water inputs to the west Fleet play a role in controlling these events, as well as the water residence time.

Chapter 4

The use of HPLC pigment signatures to assess phytoplankton assemblages in the Fleet lagoon

4.1 Introduction

During the last two decades photosynthetic pigments have proved to be extremely useful biomarkers for elucidating the composition of phytoplankton populations in diverse areas of the world oceans (Barlow et al. 1993a). More recently, characterization of pigment signatures has also been applied to estimate phytoplankton dynamics in coastal and estuarine waters (Tester et al. 1995, Pinckney et al. 1997, Ansotegui et al. 2001). This approach has proven to be useful to assess the relative importance of the more delicate or smaller components of the phytoplankton, e.g. cyanobacteria and prochlorophytes, and in the detection of fragile flagellates that do not survive fixative procedures necessary for microscopic observations (Jeffrey and Vesk, 1997).

Chromatographic separation and characterization of individual chlorophylls, chlorophyll derivatives and carotenoids provide researchers with definitive information for characterization of algal assemblages (Millie et al. 1993). Table 4.1 lists the major pigments of algal Divisions/Classes, according to Jeffrey and Vesk (1997) and Millie et al.

(1993) and can be used as a guide to taxonomic usefulness of pigments for phytoplankton ecology.

Although pigment content do vary between cells within a taxon or between taxa and with physiological state, the abundance of these diagnostic pigments generally reflects the major distribution of the respective phytoplankton groups (Millie et al. 1993). However, only a few accessory chlorophylls and carotenoids show an unambiguous chemotaxonomic interpretation. Among these, divinyl chlorophylls can be used as pigment signatures for prochlorophytes (Jeffrey and Vesk, 1997), 19'hexa for some prymnesiophytes (Jeffrey and Vesk, 1997) while peridinin is the accessory pigment characteristic of most photosynthetic dinoflagellates.

In many cases, the use of certain diagnostic pigments should be exercised with caution due to their presence in more than one algal group. Fucoxanthin, which is frequently associated with diatoms, occurs in all prymnesiophytes and is present in chrysophytes and raphydophytes (Table 4.1). 19'buta has been assigned to pelagophytes (chrysophytes), but it has also been found in some prymnesiophytes. Chlorophyll-b is present in euglenophytes, chlorophytes and prasinophytes while zeaxanthin is found in cyanobacteria, prochlorophytes, chlorophytes, cryptophytes and prasinophytes. Moreover, alloxanthin, the major carotenoid in cryptophytes has been found in the ciliate *M. rubra*, that can ingest cryptophytes and retain their organelles (Gustafson et al. 2000) and in the dinoflagellate *Dinophysis norvegica* (Meyer-Harms and Pollehne, 1998).

Among the dinoflagellates, there are some species that do not contain peridinin; some of them contain phycobilins, like some *Dinophysis* (Hewes et al. 1998), while others may contain fucoxanthin, 19'buta and/or 19'hexa, like *Gymnodinium mikimotoi* (Hansen et al. 2000), *Gymnodinium galatheanum*, *Gyrodinium aureolum* (probably =*Karenia mikimotoi*) and *Karenia brevis* (as *Gymnodinium breve*), that are the result of three sequential endosymbiotic events (Tengs et al. 2000). Moreover, other dinoflagellate species are heterotrophic and do not possess any photosynthetic pigment. Therefore, when dealing with natural communities, microscopic observations are still required to obtain a reliable interpretation of the information derived from pigment analysis (Ansotegui et al. 2001, Tester et al. 1995).

Table 4.1: Distribution of major and taxonomically significant pigments in algal divisions/classes, according to Jeffrey and Vesk (1997) and Millie et al. (1993).

Pigments	**Algal Division/Class**
Chlorophylls	
Chl-a	In all groups
Chl-b	Chlorophytes, euglenophytes, prasinophytes, prochlorophytes
Chl-c_1	Bacillariophytes, prymnesiophytes, raphidophytes
Chl-c_2	Cryptophytes, bacillariophytes, dinophytes, prymnesiophytes, chrysophytes, raphidophytes
Chl-c_3	Prymnesiophytes, chrysophytes, bacillariophytes, dinophytes
Carotenoids	
Alloxanthin	Cryptophytes
19'butanoyloxyfucoxanthin	Prymnesiophytes, chrysophytes
19'hexanoyloxyfucoxanthin	Prymnesiophytes
Diadinoxanthin	Euglenophytes, bacillariophytes, dinophytes, prymnesiophytes, chrysophytes, raphidophytes
Diatoxanthin	Euglenophytes, bacillariophytes, dinophytes, prymnesiophytes, chrysophytes, raphidophytes
Fucoxanthin	Bacillariophytes, prymnesiophytes, chrysophytes, raphidophytes
Lutein	Chlorophytes, prasinophytes
Peridinin	Dinophytes
Violaxanthin	Chlorophytes, prasinophytes, eustigmatophytes
Zeaxanthin	Cyanophytes, prochlorophytes, rhodophytes, chlorophytes
β-carotene	In all groups except rhodophytes

4.2 Results

The most abundant and recurrent pigments detected by HPLC in Fleet lagoon water samples were chl-a, chl-c_2 (although chl-c_1 and chl-c_2 are not resolved by the method used, analysis of peak absorbance spectra identified it as chl-c_2), chl-b, peridinin, alloxanthin, diadinoxanthin, β-carotene, fucoxanthin, 19'hexanoyloxyfucoxanthin (19'hexa) and diatoxanthin. Violaxanthin and zeaxanthin+lutein (not resolved by the method used and present in low concentrations, that did not allow the positive identification by absorbance spectra analysis) were observed only in 2001. Alloxanthin concentrations for the 2000 dataset and diatoxanthin concentrations during 2001 could not be calculated because the HPLC had not been previously calibrated for these pigments. However, alloxanthin was present in samples collected during 2000, as shown by the presence of a peak at the alloxanthin retention time. Based solely on peak area, alloxanthin concentrations peaked on 1/6/00 at station 1 during 2000. No peaks corresponding to chl-c_3 and prasinoxanthin were detected in samples from the Fleet. All the major pigments were identified, and only a few minor peaks in the chromatograms could not be assigned. Accessory pigment to chl-a ratios have been used to give an indication of the contribution of each pigment to total chl-a biomass.

4.2.1 *Chlorophyll-a concentrations: Comparison between HPLC and fluorometric determinations*

As previously observed for fluorescence measured chl-a, two chl-a peaks occurred during 2000 at station 1, on 1/6/00 and 19/9 - 3/10/00. However, chl-a absolute values measured by HPLC were considerably lower than values measured by fluorescence (Fig. 4.1). The fluorometer consistently overestimated chl-a values, as shown by the linear regression of fluorometer chl-a versus HPLC chl-a (Fig. 4.1), that has a slope of 3.2 and an intercept of 3.1 for the 2000 dataset (r^2=0.83, n= 81, $p < 0.01$) and slope of 2.5 and intercept of 0.3 for the 2001 dataset (r^2=0.99, n= 43, $p < 0.01$). Sigleo et al. (2000) reported a slope of 2.6 and an intercept of 0.2 (r^2=0.75, n= 26) for the linear regression of fluorometer chl-a versus HPLC chl-a using data from the Weddell-Scotia confluence. In a station close to the shore of the Gulf of Mexico, where chl-c concentrations reached locally high levels, Bianchi et al. (1995) found that chl-a was overestimated by an average 16% when measured using the standard fluorometric method, relative to the HPLC derived value.

The HPLC method is considered to be the most accurate method for measuring pigment composition (Mantoura et al. 1997). Fluorimetric methods present several limitations, including the inability to resolve chl-b and c, chlorophyll derivatives (phaeophytins, phaeophorbides, chlorophyllides and divinyl-chl-a) and the variability among *in vivo* fluorescence yields of photosystem II, due to differences in light emitted from different taxa, a taxon's physiological state, and the taxon's physiological history of light exposure (Millie et al. 1993). The errors associated with the fluorimetric method for chl-a determination are a function of the suite of pigments present in the sample. The fluorimetric method over- and underestimates chl-a and chlorophyll degradation products, respectively, when large amounts of chl-c are present within samples, while the reverse occurs when chl-b is present (Trees et al. 1985). Individual pigment measurements have indicated that chlorophyllide-a alone produces a strong signal for chl-a with the fluorometer (Trees et al. 1985). The use of HPLC minimizes the interferences caused by overlapping absorption and fluorescence bands of the various pigments since they are physically separated on the column and individually quantified by absorption and/or fluorescence detectors (Trees et al. 1985).

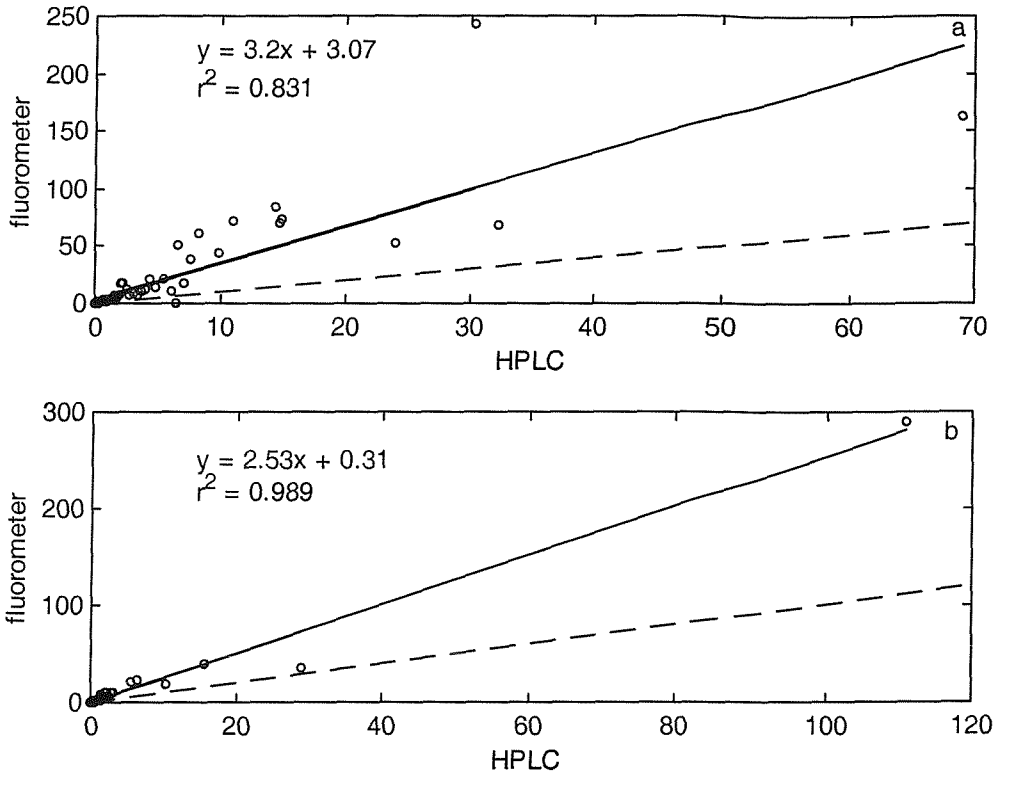


Figure 4.1: Chl-a values measured by fluorescence against chl-a measured by HPLC during (a) 2000 (n= 81, $p < 0.01$) and (b) 2001 (n= 43, $p < 0.01$) in the Fleet lagoon. The solid lines represent the trendline for each set of data and the equation for this line and correlation coefficient are shown. The dashed line represents the 1:1 agreement line.

Trees et al. (1985) reported errors that varied between - 68 to + 53 % when comparing the concentration of chl-a in 300 oceanic samples measured both fluorometrically and by HPLC. Mantoura et al. (1997) reported a correlation coefficient of 0.85 when comparing chl-a concentrations determined fluorometrically according to the method of Holm-Hansen et al. (1965) and by HPLC. The fluorometric method used in this work (Welschmeyer, 1994) optimizes a new combination of lamps and optical filters to improve the selective detection of chlorophyll-a in the presence of chlorophyll-b and phaeopigments.

The discrepancy between chl-a values determined by HPLC and fluorometrically in samples from the Fleet lagoon seems to be magnified at high chl-a values. On the two days of peak chl-a values due to high abundances of cryptophytes, 1/6/00 and 6/4/01, the HPLC results were 69 and 111 μg chl-a L^{-1} respectively, while the fluorometer indicated 163 and 290 μg chl-a L^{-1}, both values being around 250 % overestimated. The other exceptionally high chl-a value observed in the Fleet, as indicated by the fluorescence method, occurred on 19/9/00 at station 1 during the bloom of *Prorocentrum micans*, and this time the flurometer determined chl-a was 244 μg chl-a L^{-1}, while the HPLC result was

only 30 μg chl-a L^{-1}. On the following sampling date, on 3/10/00, the fluorometer indicated 68 μg chl-a L^{-1} and the HPLC 32 μg chl-a L^{-1}. *P. micans* is a swimming dinoflagellate that tends to accumulate at the water surface in the sample bottle. It is possible that on 19/9/00 the sample for chl-a determination by fluorimetry was not properly homogenized, resulting in an extremely high chl-a value. However, phytoplankton cell counts also showed very high *P. micans* abundances at station 1 on this date (43623 cells mL^{-1}) and the concomitant occurrence of spring tides and a decreased chl-a value at station 2 indicates that the *P. micans* population was concentrated towards Abbotsbury on this day, and it is likely that extremely high chl-a values have resulted from this process.

Increased phaeopigment (phaeophorbides + phaeophytin) concentrations were observed from 5/9/00 through 19/9/00 and particularly in 3/10/00 at station 1 and it is possible that they influenced the discrepancy observed between the chl-a values measured by the two methods during this period. Moreover, higher phaeophorbide concentrations were also observed at stations 1, 3 and 4 on 27/11/01, and the fluorometer consistently overestimated chl-a concentrations in these samples by 27, 29 and 54 %. Intriguingly, chl-c and chl-b concentrations were low relative to chl-a in the Fleet during most of the period studied and therefore are not expected to interfere considerably on the measurement of chl-a by fluorescence.

4.2.2 *Temporal changes year 2000*

Temporal changes of HPLC determined chl-a followed trends described previously for fluorometrically determined chl-a (see Fig. 3.15a), although absolute values varied (Fig. 4.2a). Chl-c_2 and β-carotene displayed a similar seasonal trend to chl-a, with maxima at station 1 of 6.5 μg L^{-1} and 4.9 μg L^{-1} on 1/6/00 followed by 1.9 μg L^{-1} and 1.7 μg L^{-1} on 3/10/00 respectively (Fig. 4.2g, e). These two pigments showed considerably lower, frequently close to zero, values at stations 6 and 7. At stations 2 to 5 peak concentrations of chl-c_2 and β-carotene were respectively 0.99 μg L^{-1} on 5/9/00 and 0.28 μg L^{-1} on 11/7/00.

Concentrations of diadinoxanthin closely followed those of peridinin, and both pigments peaked at station 1 on 19/9/00 (Fig. 4.2b, c). At station 2 the same temporal trend already observed for chl-a, of two high values on 5/9 and 3/10/00 with a lower one in between on 19/9/00, was observed for these two pigments. Maximum values of peridinin and diadinoxanthin were observed at stations 3 to 5 on 17/10/00. At stations 6 and 7 these pigments peaked on 4/11/00 while concentrations were close to zero during

the rest of the year. Diatoxanthin concentration peaked at station 1 just after peridinin and diadinoxanthin, on 3/10/00, when 1.8 μg L^{-1} was measured (Fig. 4.2h). At station 2 this pigment peaked on 11/7/00, at a concentration of 0.98 μg L^{-1}.

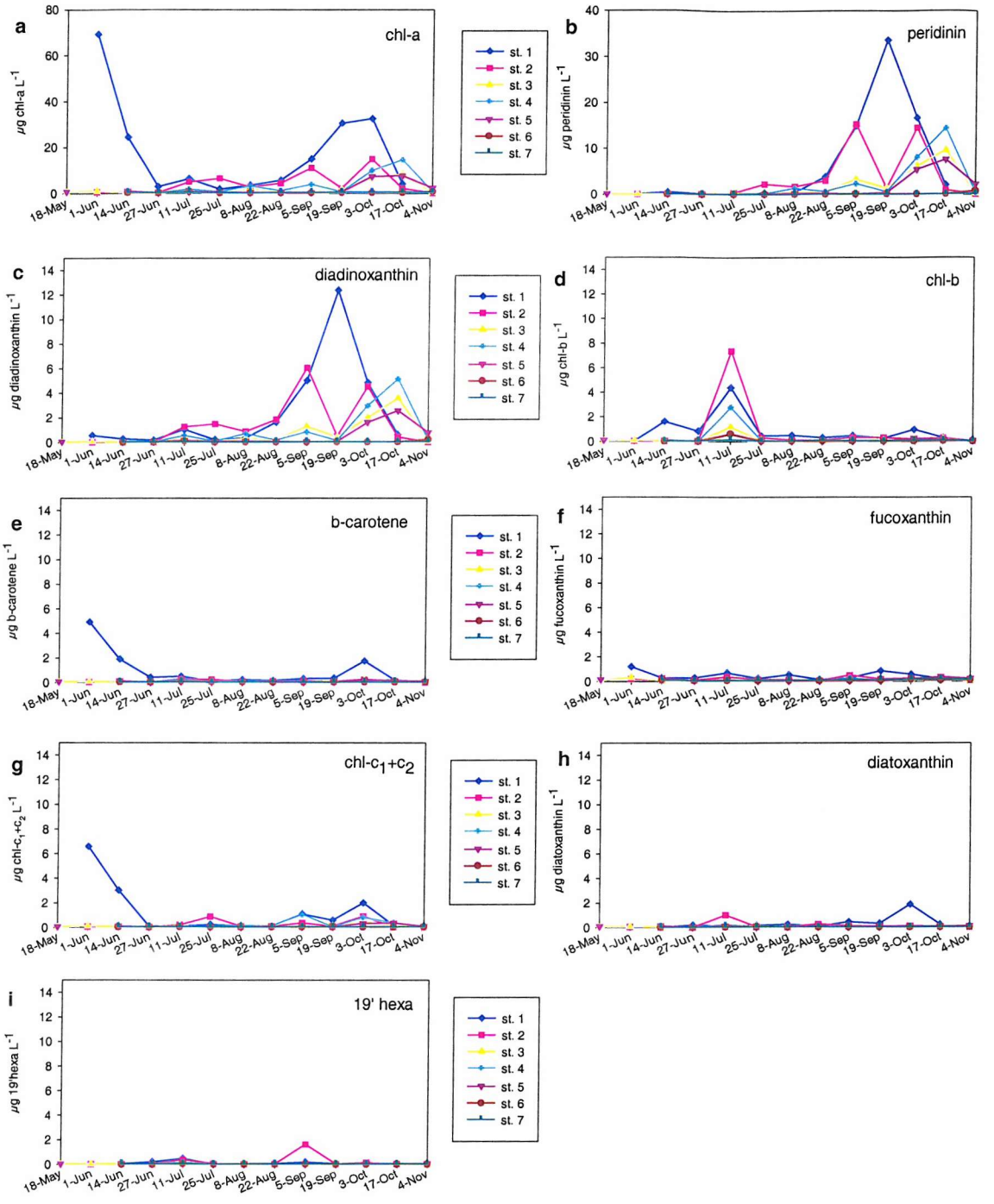


Figure 4.2: Pigment concentrations at seven stations along the Fleet lagoon during 2000. a) chlorophyll-a, b) peridinin, c) diadinoxanthin, d) chlorophyll-b, e) β-carotene, f) fucoxanthin, g) chlorophyll-c_1+c_2, h) diatoxanthin, i)19'hexa.

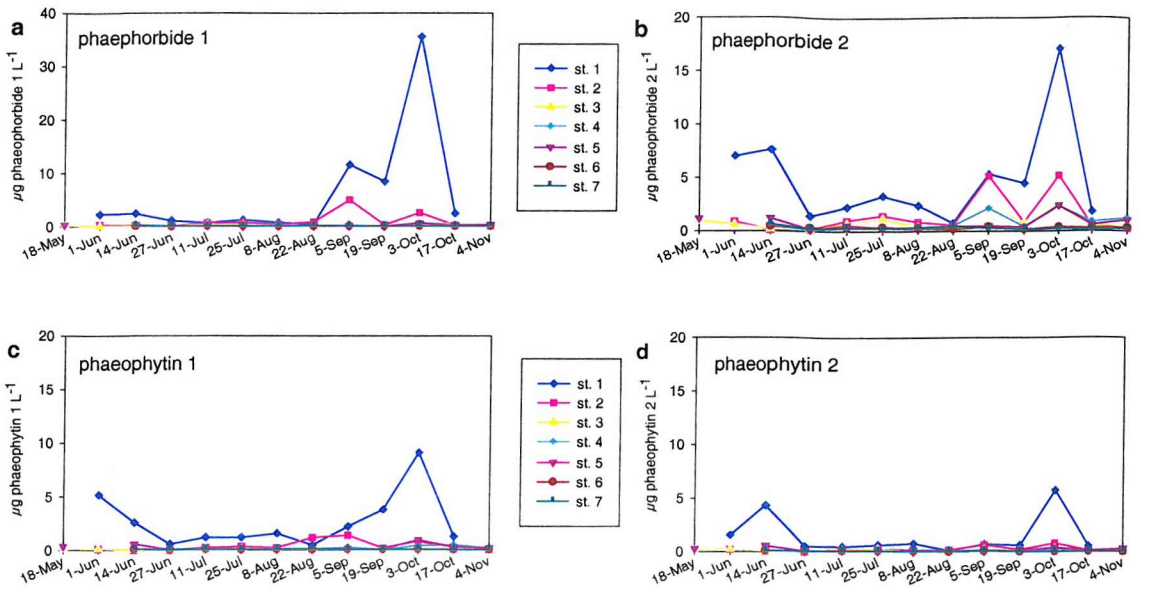


Figure 4.3: Phaeopigment concentrations at seven stations along the Fleet lagoon during 2000. a) phaeophorbide 1, b) phaeophorbide 2, c) phaeophytin 1, d) phaeophytin 2.

Chl-b showed a different seasonal pattern, peaking along the whole lagoon on 11/7/00, with maxima at station 1 of 4.4 μg L^{-1}. At station 1 two other smaller peaks were observed on 14/6/00 (1.6 μg L^{-1}) and 3/10/00 (0.9 μg L^{-1}) (Fig. 4.2d). This pigment was often not detectable in samples from stations in the mid Fleet.

Fucoxanthin concentration was low throughout the whole lagoon. The peak value at station 1 was only 1.2 μg L^{-1} on 1/6/00, while at station 7 the maximum value observed was 0.17 μg L^{-1} on 17/10/00 (Fig. 4.2f).

Increased amounts of 19'hexa were measured on 11/7/00 at stations 1 to 6 (peak value of 0.44 μg L^{-1} at station 1) and particularly on 5/9/00 at station 2 when 1.60 μg L^{-1} was measured (Fig. 4.2i). Extremely low values of 19'hexa (0.01 μg L^{-1}) were measured occasionally at stations 6 and 7, but most of the time this pigment was not detected.

Phaeophorbide 1 and 2 and phaeophytin 1 and 2 showed a similar trend. All these pigments peaked on the 3/10/00 at station 1, when high values were observed, of 35.5 μg L^{-1} of phaeophorbide 1, 17.0 μg L^{-1} of phaeophorbide 2, 9.08 μg L^{-1} of phaeophytin 1 and 5.70 μg L^{-1} of phaeophytin 2 (Fig. 4.3). Phaeophorbide 2 and phaeophytin 1 and 2 were also high in June at station 1. These pigments were consistently observed at this station. Considerably lower phaeopigment levels were observed in the east relative to the west Fleet. At stations 6 and 7 phaeophytins were generally lower than 0.1 μg L^{-1}.

Considering accessory pigment to chl-a ratios, the peridinin to chl-a ratio showed consistently high values from late July, and was above 1, and up to 1.4 initially at stations

1 to 4 in September and later also at stations 5 to 7 in November (Fig. 4.4a). These high peridinin to chl-a ratios were consistent with high biomass of *P. micans*, that developed inside the lagoon and then moved eastwards during October (see chapter 3).

Diadinoxanthin to chl-a ratios followed the same temporal pattern as peridinin to chl-a ratios, indicating that dinoflagellates were the main group associated with this pigment in the Fleet (Fig. 4.4b). On 11/7/00 it is possible that *Eutreptiella marina* contributed to the increased diadinoxanthin/chl-a ratios at stations 2 to 4 (up to 0.32), as peridinin/chl-a ratios were low at these stations on this date (0.07-0.08), except at station 3, where it was 0.26, and was associated with *P. micans* abundances of 195 cells mL^{-1}.

Chl-b/chl-a ratios were lower than 0.21 during the whole year except for the period from 27/6/00 to 25/7/00 (Fig. 4.4c). Peak values occurred on 11/7/00 throughout the whole lagoon, but particularly at stations 2 and 4, where this ratio was 1.50 and 1.66 and coincided with high abundances of *E. marina.*

Chl-c_2/chl-a ratios were low during most part of the year and reached peak values of 0.17 occasionally during June and July (Fig. 4.4d). 19'hexa was only occasionally detected and ratios of this pigment to chl-a were generally low (Fig. 4.4e). β-carotene to chl-a ratios were in general lower than 0.16, except on 25/7/00 at station 6, when this ratio was 0.54 (Fig. 4.4f). Relatively higher values were consistently found at station 6.

Diatoxanthin to chl-a ratios varied between zero and 0.2 and were in general higher in the mid Fleet (Fig. 4.4g). From 8/8/00 to 17/10/00, during the *P. micans* bloom in the inner Fleet, this ratio presented consistently low values. Fucoxanthin to chl-a ratios were quite variable in the Fleet during 2000. In general, lower values (up to 0.15 on 8/8/00) were observed at station 1, while higher values were found at stations 6 and 7 (up to 0.49), although quite high values were also observed at stations 2, 4 and 5 on 27/6/00 (Fig. 4.4h). Increased fucoxanthin/chl-a ratios in the west Fleet indicate the greater contribution of diatoms to the total phytoplankton biomass at the marine end of the lagoon.

Phaeophorbides 1 and 2 to chl-a ratios were quite variable and were high on some occasions, with station 6 showing consistently higher values (Fig. 4.5a, b). On 25/7/00, chl-a at station 6 was extremely low, only 0.08 $\mu g\ L^{-1}$ and it is possible that this was underestimated, thus explaining the very high phaeopigments/chl-a ratios on this date. At station 1, phaeophorbide 1/chl-a ratio was generally low, although high values were observed on 25/7/00, 5/9/00 and 3/10/00. Station 4 showed consistently lower phaeophorbide 1/chl-a ratios, except for the value on 4/11/00. Phaeophorbide 2/chl-a ratio was particularly high on 14 and 27/6/00 at stations 3 to 7. On 25/7/00 extremely high values of this ratio were observed at stations 1, 3 and 6, of 1.9, 3.2 and 4.3 respectively.

Later in the year values over 1 were observed in the east Fleet on 5/9/00 and in the mid Fleet on 4/11/00.

Phaeophytin 1 and 2/chl-a ratios were lower than the phaeophorbides 1 and 2 to chl-a ratios (Fig. 4.5c, d). Higher values, up to 1, were consistently found at station 6, although station 1 showed high phaeophytin 1/chl-a ratios in late July, early August, and stations 5 and 7 also showed occasional high values. Stations 2 and 4 presented lower values of this ratio.

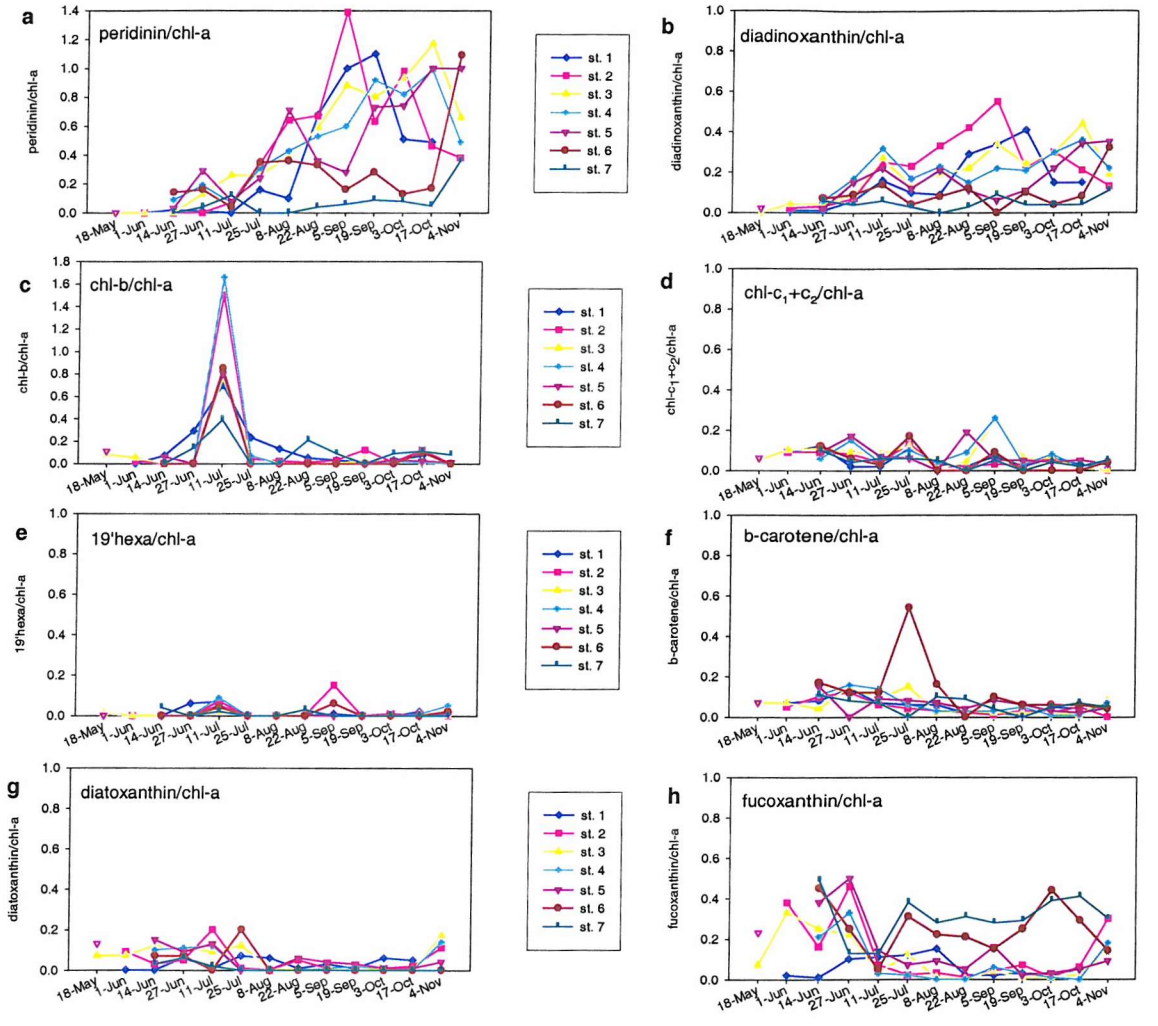


Figure 4.4: Accessory pigment/chl-a ratios during 2000 at the Fleet lagoon. a) peridinin/chl-a, b) diadinoxanthin/chl-a, c) chl-b/chl-a, d)ch-c_1+c_2/chl-a, e) 19'hexa/chl-a, f) β-carotene/chl-a, g) diatoxanthin/chl-a, h) fucoxanthin/chl-a.

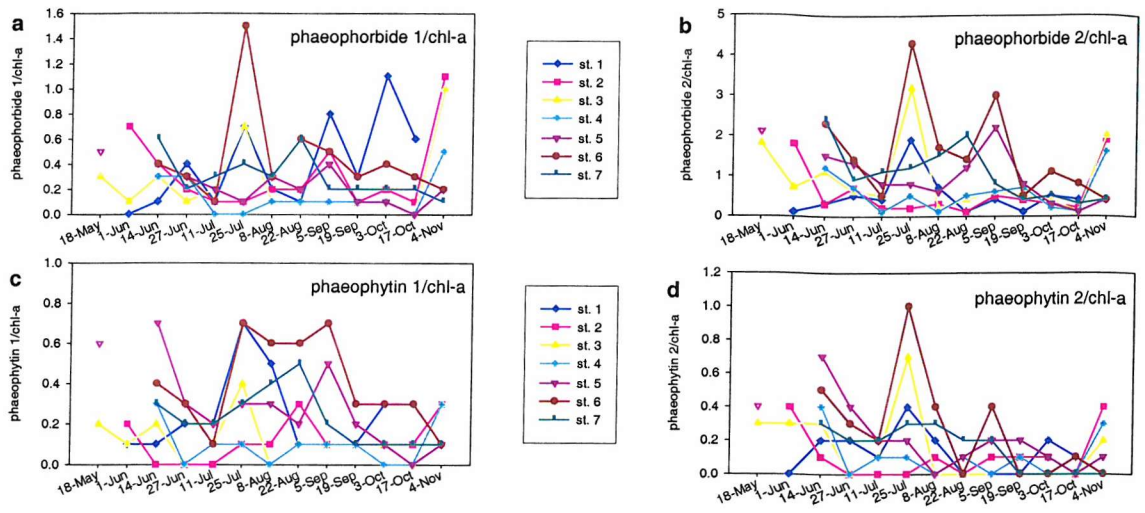


Figure 4.5: Phaeopigment/chl-a ratios during 2000 at the Fleet lagoon. a) phaeophorbide 1/chl-a, b) phaeophorbide 2/chl-a, c) phaeophytin 1/chl-a, d) phaeophytin 2/chl-a.

4.2.3 *Temporal changes year 2001*

As for the year 2000, HPLC determined chl-a followed similar trends in 2001 to those described previously for fluorimetrically determined chl-a, although absolute values were different (Fig. 4.6a). Chl-c_2, β-carotene and zeaxanthin+lutein showed a similar temporal trend as chl-a, although the increase in concentrations observed from October to November in the two latter pigments was more pronounced than the increase in chl-a and chl-c_2 (Fig. 4.6g, e, h). These pigments peaked on 6/4/01 at stations 1 and 2, on 14/6/01 at station 1 and on 27/11/01 at stations 1, 3 and 4. Fucoxanthin exhibited a pronounced peak at station 1 on 14/6/01 (12.4 μg L^{-1}), in contrast with the generally low concentrations (lower than 1.4 μg L^{-1}) observed of this pigment along the whole lagoon from April to October 2001 (Fig. 4.6f). On 27/11/01 higher values of fucoxanthin were present along the whole Fleet, except for station 2, where values were similar to the ones measured in October.

Peridinin and diadinoxanthin peaked at station 1 on 6/4/01, when values were 21.1 μg L^{-1} and 12.1 μg L^{-1} respectively. As for 2000 these pigments co-varied temporally, except for a smaller diadinoxanthin peak which was observed at station 1 on 14/6/01 (1.95 μg L^{-1}) without any associated peak of peridinin. Throughout the rest of the year peridinin concentration was close to zero along the entire lagoon, and only in August at station 5, 0.2 μg L^{-1} of this pigment was detected (Fig. 4.6b).

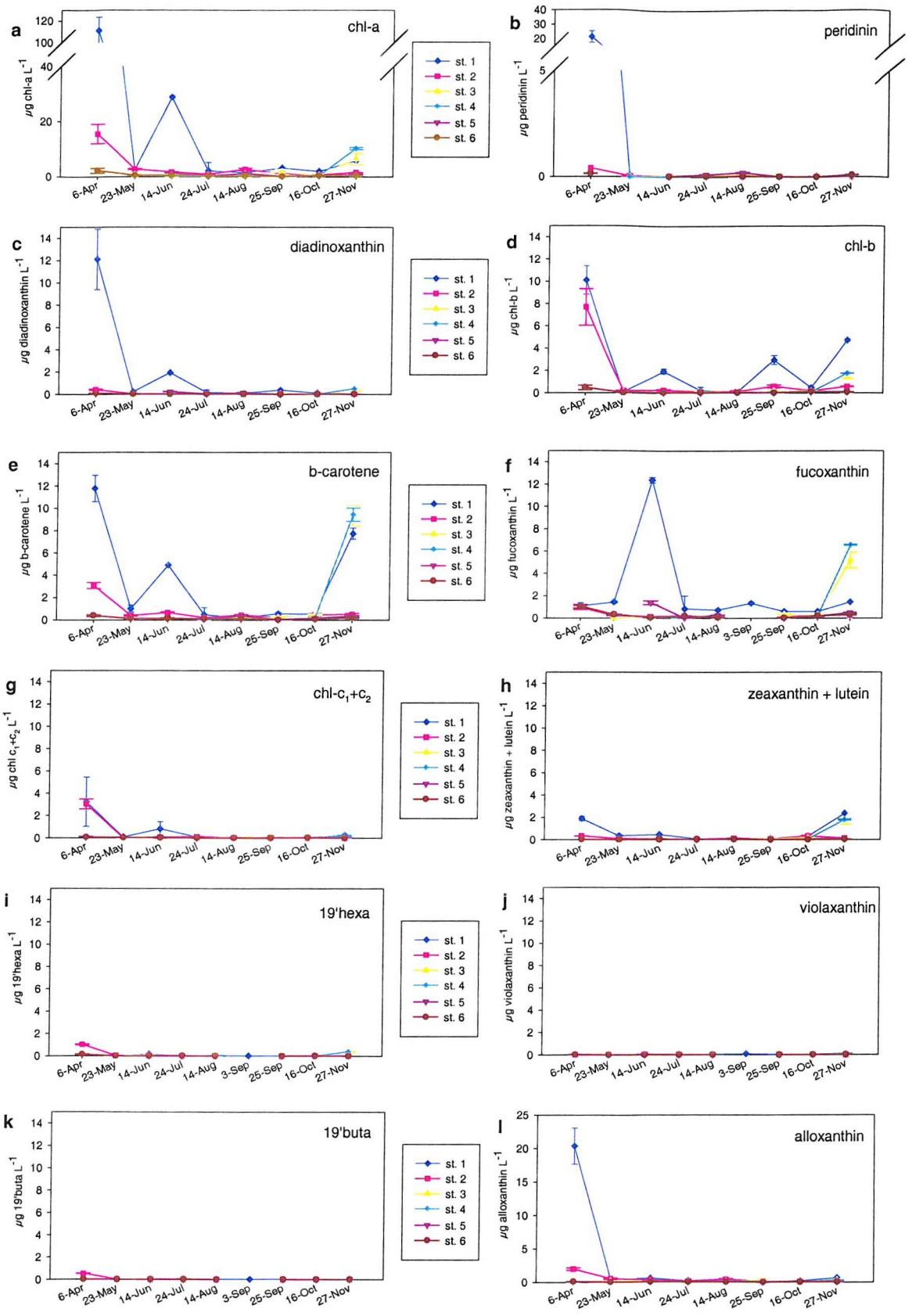


Figure 4.6: Pigment concentrations at six stations along the Fleet lagoon during 2001. a) chlorophyll-a, b) peridinin, c) diadinoxanthin, d) chlorophyll-b, e) β-carotene, f) fucoxanthin, g) chlorophyll-c_1+c_2, h) zeaxanthin+lutein, i)19'hexa, j) violaxanthin, k) 19'buta, l) alloxanthin.

Chl-b maximum concentrations were measured on 6/4/01 at stations 1 and 2 of 10.1 μg L^{-1} and 7.68 μg L^{-1} respectively (Fig. 4.6d). Several other smaller peaks were measured at station 1 on 14/6/01 (1.94 μg L^{-1}), 25/9/01 (2.94 μg L^{-1}) and 27/11/01 (4.72 μg L^{-1}). At stations 3 to 5 concentrations were lower than 0.17 μg L^{-1} until October, then increased in November. At station 6, chl-b peaked on 6/4/01 (0.47 μg L^{-1}) and was lower than 0.11 μg L^{-1} later in the year.

An extremely high alloxanthin concentration was detected on 6/4/01 at station 1 (20.3 μg L^{-1}) whereas at station 2, 1.96 μg $L^{-1}$of alloxanthin was measured on this date (Fig. 4.6l). At these stations alloxanthin was always present throughout the year, and varied between 0.05 and 0.59 μg L^{-1} from May to November. Lower values were observed in the east Fleet, particularly at stations 5 and 6, although alloxanthin was constantly detected throughout 2001. Some peaks in alloxanthin were also seen in samples from 2000 but these could not be quantified.

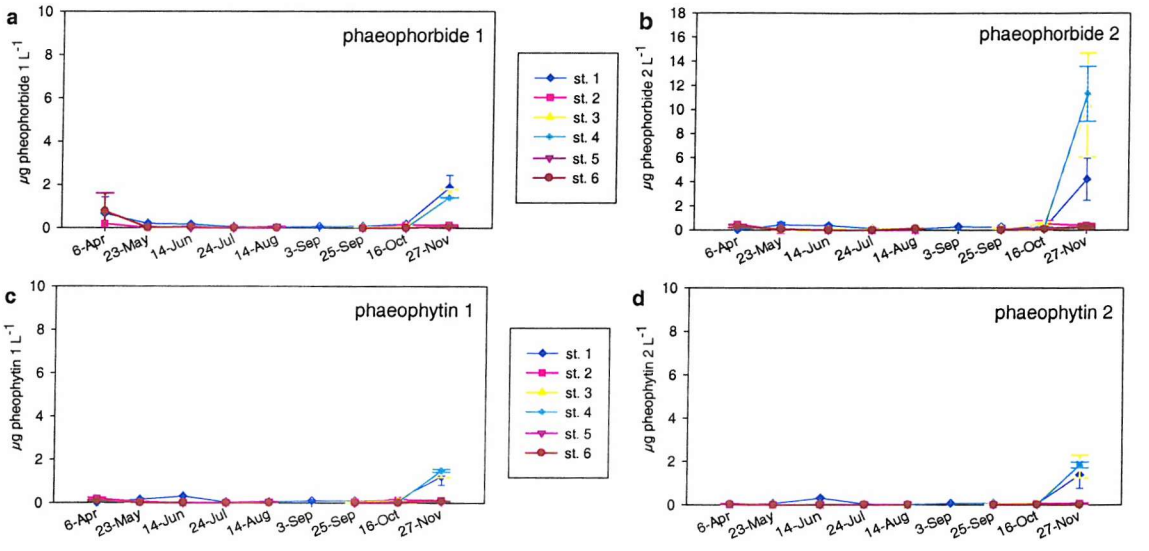


Figure 4.7: Phaeopigment concentrations at seven stations along the Fleet lagoon during 2001. a) phaeophorbide 1, b) phaeophorbide 2, c) phaeophytin 1, d) phaeophytin 2.

19'hexa was generally present at levels below 0.4 μg L^{-1} (Fig. 4.6i). This pigment peaked at station 2 on 6/4/01, when 1.0 μg L^{-1} was detected. Concentrations then decreased considerably and were close to zero throughout May to October, increasing in November at stations 3 and 4 where 0.37 μg L^{-1} were measured.

Low concentrations of 19'buta and violaxanthin were observed in the Fleet during 2001 (Fig. 4.6k, j). These pigments were not detected in samples collected during 2000. 19'buta was present at station 2 on 6/4/01 at 0.51 μg L^{-1}, and after that zero or close to

zero values were detected in the whole Fleet. Violaxanthin concentrations varied between zero and 0.06 μg L^{-1} (Fig. 4.6j).

Phaeophorbide 1 was found in high concentrations in samples from April and November 2001, maxima of 0.78 μg L^{-1} and 1.87 μg L^{-1}, respectively (Fig. 4.7a). Phaeophorbide 2 concentrations were higher relative to phaeophorbide 1, particularly at station 1. On 27/11/01 high values of phaeophorbide 2 were measured at stations 1, 3 and 4, of 4.2 μg L^{-1}, 10.4 μg L^{-1} and 11.3 μg L^{-1} respectively (Fig. 4.7b). Phaeophorbide 1 and phaeophytin 1 and 2 concentrations also peaked in November at stations 1, 3 and 4. Concentrations of these pigments were higher at station 1 throughout the year and were close to zero at other stations (Fig. 4.7).

Considering the accessory pigment to chl-a ratios, peridinin/chl-a ratio showed much lower values than in the previous year. This ratio peaked at station 5 on 24/7/01 (Fig. 4.8a), when it was 0.50, coinciding with abundances of 163 cells mL^{-1} of an athecate dinoflagellate species and 16 cells mL^{-1} of *P. micans*. At stations 1 to 4, where high values of this ratio were observed in autumn 2000, lower values (maximum of 0.19) were observed in 2001 and station 6 showed a higher peridinin to chl-a ratio throughout 2001, up to 0.26.

Diadinoxanthin/chl-a ratios were quite constant throughout 2001, around 0.1, and peaked at station 5 on 14/6/01 and 24/7/01 at 0.20 (Fig. 4.8b). Chl-b/chl-a ratio ranged between 0.03 and 0.96 and was on average 0.21 (Fig. 4.8c). Maximum values were observed at station 1 in September and November. Chl-c_2/chl-a ratio peaked at station 5 in September (0.62), but was lower than 0.20 during the rest of the year (Fig. 4.8d).

Alloxanthin/chl-a ranged between 0.02 and 0.25 throughout 2001. At station 6 this ratio was quite constant around 0.08 during most of the period studied (Fig. 4.8e). β-carotene/chl-a peaked in the whole lagoon, except at station 2, in November 2001 (Fig. 4.8f). Maximum values of 1.52 and 1.37 were found at stations 3 and 1 respectively. From April to September this ratio was lower than 0.55.

Zeaxanthin+lutein/chl-a ratios showed an increase towards October and November in most parts of the lagoon, reaching a peak of 0.53 at station 2 in October (Fig. 4.8g). Fucoxanthin/chl-a ratio was higher at stations 5 and 6, although station 1 showed considerable fucoxanthin contribution, in contrast to the year 2000. Stations 2, 3 and 4 presented relatively lower fucoxanthin/chl-a values during most of the period studied, and peak values occurred at these stations in November (Fig. 4.8h). 19'buta, 19'hexa and violaxanthin to chl-a ratios were close to zero during most part of 2001 (Fig. 4.8i, j, k) with maximum values of these ratios respectively 0.09, 0.13 and 0.07.

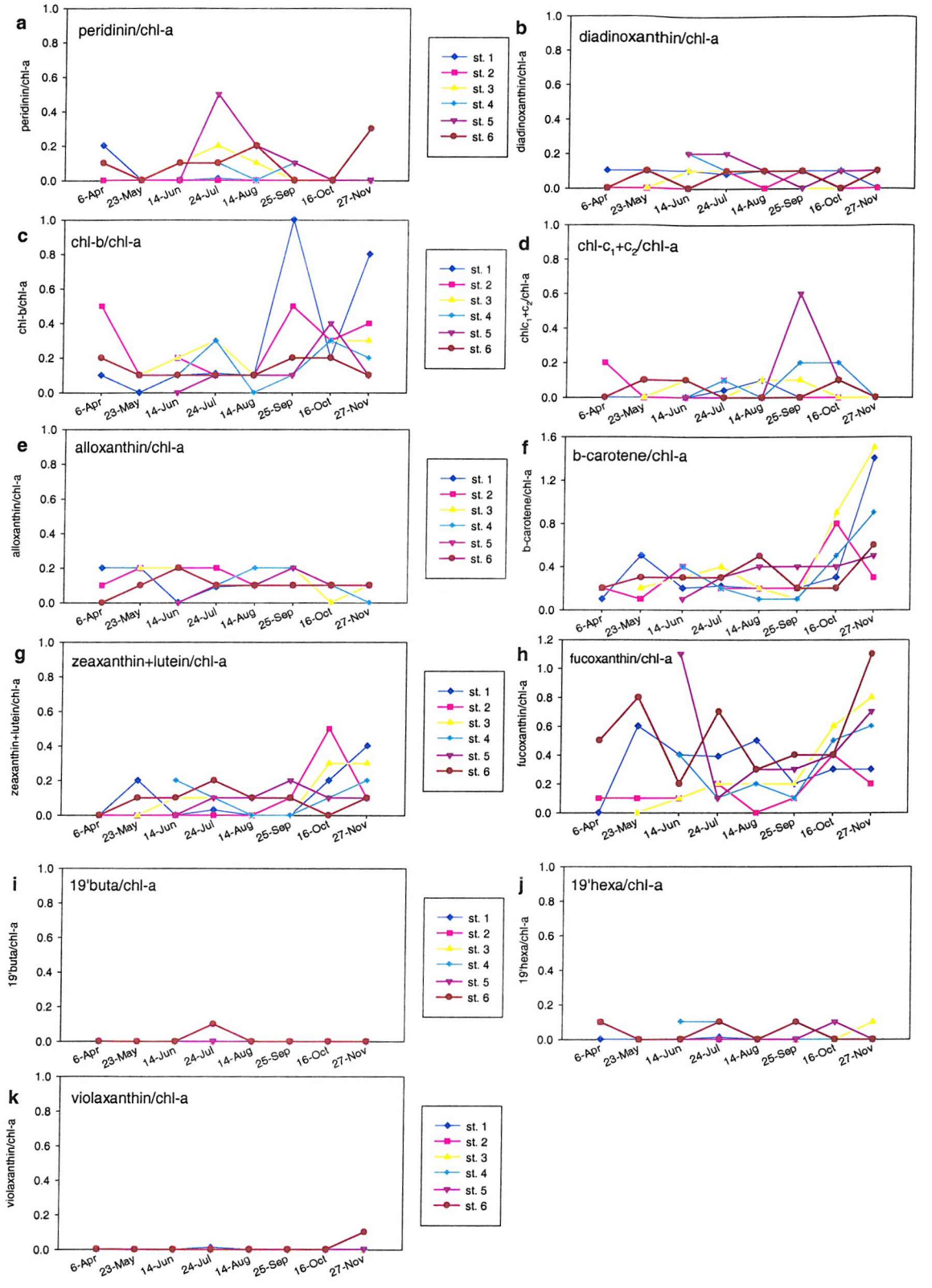


Figure 4.8: Accessory pigment/chl-a ratios at six stations along the Fleet lagoon during 2001. a) peridinin/chl-a, b) diadinoxanthin/chl-a, c) chl-b/chl-a, d)ch-c_1+c_2/chl-a, e) alloxanthin/chl-a, f) β-carotene/chl-a, g) zeaxanthin+lutein/chl-a, h) fucoxanthin/chl-a, i) 19'buta/chl-a, j) 19'hexa/chl-a, k) violaxanthin/chl-a.

In contrast to 2000, phaeophorbides 1 and 2 to chl-a and phaeophytin 1 and 2 to chl-a ratios were quite low during most of 2001 (Fig. 4.9). These ratios, and particularly the phaeophorbide 2/chl-a ratio considerably increased in October and November and a peak value of 1.60 was found at station 3 in November. Phaeophorbide 1/chl-a ratio was also high (0.50) in April 2001 at station 6.

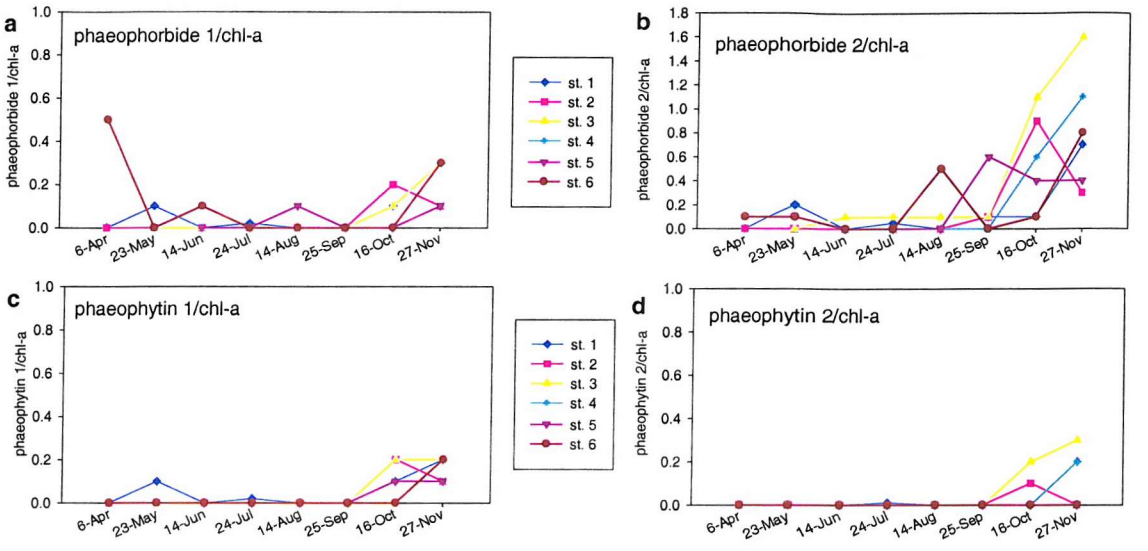


Figure 4.9: Phaeopigment/chl-a ratios at six stations along the Fleet lagoon during 2001. a) phaeophorbide 1/chl-a, b) phaeophorbide 2/chl-a, c) phaeophytin 1/chl-a, d) phaeophytin 2/chl-a.

4.4 Discussion

Most studies of HPLC determined phytoplankton pigments have been performed in the open ocean (Everitt et al. 1990, Barlow et al. 1993a, Letelier et al. 1993, Brunet et al. 1996, Breton et al. 2000, Sigleo et al. 2000, Garibotti et al. 2003). There are only a few studies reporting on phytoplanktonic pigment composition in estuarine and coastal waters, and no other reported results using this technique in coastal lagoons. Fucoxanthin has been reported as the dominant accessory pigment in the Urdaibai estuary, where it was attributed to diatoms (Ansotegui et al. 2001) and in the Newport river estuary, where it was associated with chrysophytes and prymnesiophytes (Tester et al. 1995). In the Neuse river estuary, pigments were variable within and among sampling sites, and a more diverse pigment composition, including fucoxanthin, peridinin, alloxanthin, chl-b and zeaxanthin was reported (Pinckney et al. 1997).

In samples from the Fleet lagoon, microscopic observations revealed that the peaks of chl-a and those of alloxanthin (in 2000 dataset inferred by the large peak area) at station 1 in June 2000 and April 2001 were associated with high biomasses of cryptophytes (see

chapter 3), of 1242 and 10218 μg C L^{-1} respectively (Fig. 4.10a). Another alloxanthin-containing organism also observed in high abundance in samples from the Fleet, particularly during 2001 was the ciliate *M. rubra*. High biomass of this photoautotrophic ciliate was observed at station 2 on 14/8/01 (818 μg C L^{-1}), together with cryptophytes; therefore it is possible that this ciliate has contributed to the alloxanthin value of 0.38 μg L^{-1} measured on this day.

Increased amounts of chl-c_2, of 6.54 μg L^{-1} and 3.23 μg L^{-1} observed at station 1 on 1/6/00 and 6/4/01 are likely to be due to high densities of cryptophytes. β-carotene concentration was also high at station 1 on those dates (4.87 μg L^{-1} and 11.77 μg L^{-1}). This pigment has primarily a protective role of the photosynthetic apparatus under conditions of high or potentially damaging light.

On 11/7/00, high chl-b concentrations, up to 7.34 μg L^{-1}at stations 1 to 6 were associated with a high *E. marina* biomass, up to 1008 μg L^{-1}at station 2 (Fig. 4.10b). However, even higher *E. marina* biomass (3139 μg L^{-1}) was observed at station 1 on 1/6/00, but no chl-b was measured on this day. At station 1 on 25/9/01 and 27/11/01, chl-b concentrations of 2.94 μg L^{-1} and 4.72 μg L^{-1} respectively were associated with *E. marina* biomass of 323 and 115 μg C L^{-1}.

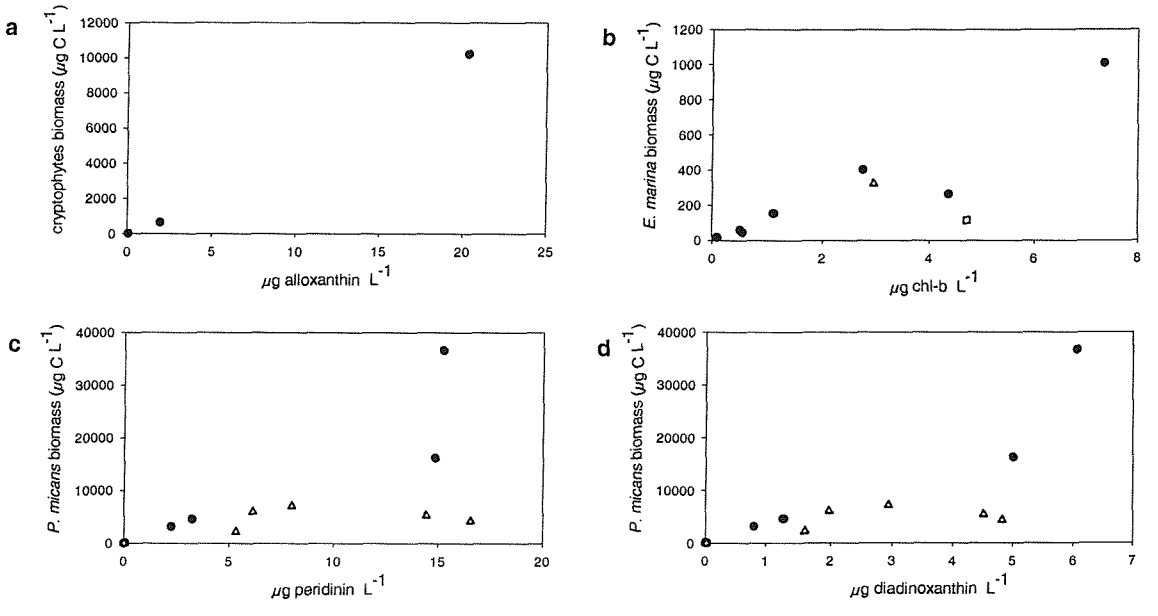


Figure 4.10: Biomass (μg C L^{-1}) of certain phytoplankton species versus the concentration of its major pigment biomarker on selected dates in the Fleet lagoon. a) alloxanthin versus cryptophytes on 6/4/01, b) chl-b versus *Eutreptiella marina* on 11/7/00 (filled circles), on 15/9/01 (open triangle) and on 27/11/01 (open square), c) peridinin and *Prorocentrum micans* on 5/9/00 (circles) and 3/10/00 (open triangles) and d) diadinoxanthin and *P. micans* on 5/9/00(circles) and 3/10/00 (triangles).

Peridinin was the prominent accessory pigment during 2000. High levels of this pigment occurred at stations 1 and 2 from late August to early October 2000 (up to 33.46 μg L^{-1} on 19/9/00) and later in the year at stations 3 to 7. This temporal pattern agreed reasonably well with the exceptional high *P. micans* biomass initially observed in the inner Fleet, that peaked at station 1 on 19/9/00 (62298 μg C L^{-1}) and was later flushed out of the lagoon (see chapter 3) (Fig. 4.10c). Diadinoxanthin concentrations closely matched the variations of peridinin levels, although absolute values were lower (up to 12.35 μg L^{-1} on 19/9/00), indicating that this pigment is also related to the *P. micans* bloom (Fig. 4.10d). Fig. 4.10 shows that there is a reasonable relation between biomarker pigment concentrations and the biomass of associated species.

The xanthophylls diadinoxanthin and diatoxanthin function as photoprotective pigments in chromophyte algae. When algae are exposed to high irradiance, some excess energy is dissipated by the interconversion of diadinoxanthin into diatoxanthin, referred to as the xanthophyll cycle (Porra et al. 1997, Fujiki et al. 2003). Diatoxanthin peaked on 3/10/00 at station 1, when 1.83 μg L^{-1} of this pigment was measured. A small peak in β-carotene (1.72 μg L^{-1}) was also observed on this date. However, it is also possible that the increased diatoxanthin levels on 3/10/00 indicate the senescence of the *P. micans* population, as in laboratory experiments with *P. lima*, diatoxanthin levels increased towards the stationary growth phase, although light conditions remained constant (see chapter 6).

In July and early August 2000, considerable biomass of the dinoflagellates *Scrippsiella* spp., athecates of 20 and 30 μm in length and an unidentified thecate species, as well as *P. micans* were observed at stations 1 to 4, but peridinin concentrations remained relatively low throughout this period. Slightly increased levels of peridinin during this period were associated with higher biomass of *P. micans* relative to the other species. Differences between the cellular pigment content among different species will account for these discrepancies. Laboratory and field studies have shown that the ratios of individual accessory pigments to chl-a can vary as a function of taxonomic composition and physiological state, as modulated by nutrients, temperature, light intensity and spectral composition and photoperiod (Millie et al. 1993, Henriksen et al. 2002).

Moreover, some *Gymnodinium* species (athecate forms) do not have peridinin, but possess an anomalously pigmented plastid, in which the carotenoid composition is dominated by fucoxanthin, 19'hexa and 19'buta (Hansen et al. 2000, Tengs et al. 2000). In July 2001 high biomass of *Scrippsiella* spp. were again observed, with no associated peridinin. This time *Scrippsiella* spp. was the only autotrophic dinoflagellate species

observed. In contrast to what happened in late summer and autumn 2000, during this time in 2001 peridinin and diadinoxanthin were not detected. This is consistent with the microscopic observation of the absence of *P. micans* and other autotrophic dinoflagellate species during this period.

Fucoxanthin concentrations were quite low in the Fleet, reflecting the low biomass of diatoms in the lagoon. During 2000, the fucoxanthin to chl-a ratio was higher at station 7, due to the greater contribution of diatoms to the total phytoplankton biomass at the marine end of the lagoon. In 2001, fucoxanthin levels were mostly comparable to those detected in 2000, overall between 0 and 0.81 μg L^{-1} in 2000 and 0 and 1.43 μg L^{-1} in 2001. However, peak values of 12.36 μg L^{-1} at station 1 on 14/6/01 and 5.21 and 6.57 μg L^{-1} at stations 3 and 4 on 27/11/01 were also found. The peak of fucoxanthin on 14/6/01 is difficult to explain, as there were very few diatoms present in this sample and nanoflagellates were not observed. Although there are some dinoflagellates that produce fucoxanthin, the dominant species on this day was a heterotrophic dinoflagellate, *Cryptechodinium* cf. *cohnii*. On 27/11/01 some pennate diatoms were observed at stations 3 and 4, and are probably associated with the fucoxanthin concentrations measured.

Some inconsistencies between pigment signatures and phytoplankton species present were observed, particularly in the sample from station 1 on 6/4/01. Although quite high levels of peridinin (21.07 μg L^{-1}), diadinoxanthin (12.1 μg L^{-1}) and chl-b (10.09 μg L^{-1}) were measured in this sample, dinoflagellate abundance was only 7 cells mL^{-1}, and could not account for the high levels of the former two pigments. In addition, among the chl-b containing groups, the euglenophyte *Eutreptiella marina* was the only one previously observed along the lagoon, but this species was not present in this sample, that was characterized by an extremely high abundance of cryptophytes (425734 cells mL^{-1}).

Chl-b is also present in other algal groups such as chlorophytes, prasinophytes and prochlorophytes (Table 4.1). There are no references in the literature reporting the presence of prochlorophytes in estuarine environments (Ansotegui et al. 2001), although this group is common in the open ocean (Letelier et al. 1993). In samples from the Fleet lagoon, nanoflagellates (< 10 μm) were not identified, but were grouped and counted together. These flagellates could belong mostly to chlorophytes, prasinophytes or prymnesiophytes. In general, light microscopy is considered inaccurate for the study of small forms, in particular phytoflagellates, due to problems in sample processing as well as cell enumeration and sizing (Garibotti et al. 2003 and references therein). Cryptophytes were so numerous in the sample from station 1 on 6/4/01 that they obscured smaller cells that might have been present. However, dinoflagellates were definitely not overlooked in

this sample, and it is therefore difficult to explain why peridinin and diadinoxanthin levels were so high. Garibotti et al. (2003) and Henriksen et al. (2002) have recently reported major discrepancies between peridinin-estimated dinoflagellate biomass and carbon-estimated dinoflagellate biomass.

At station 2 on 6/4/01, the presence of 19'buta (0.51 μg L^{-1}) and 19'hexa (1.01 μg L^{-1}) may suggest that prymnesiophytes were present. The presence of chl-b (7.68 μg L^{-1}) in this sample in the absence of *E. marina* may indicate that small chlorophytes could also be present in this sample which had nanoflagellate abundance of 551 cells mL^{-1}.

19' hexa concentrations were in general lower than 0.50 μg L^{-1} during both years. On 5/9/00, 1.60 μg L^{-1} was detected at station 2 coinciding with 10 μg C L^{-1} (833 cells mL^{-1}) of nanoflagellates, suggesting that these were prymnesiophytes.

At stations 3 and 4 on 27/11/01, 19'hexa (0.36 μg L^{-1} and 0.37 μg L^{-1}) and zeaxanthin+lutein (1.64 μg L^{-1} and 1.79 μg L^{-1}) presence may indicate that prymnesiophytes and chlorophytes or cyanophytes (assuming zeaxanthin was the pigment present) occurred in low numbers. Nanoflagellates were not present in these samples.

Filamentous cyanobacteria were occasionally observed in low numbers at stations 2, 3 and 4 during both years, but zeaxanthin+lutein was not detected during 2000 and increased zeaxanthin+lutein concentrations during 2001 did not directly match increased apparent abundances of this organism. It is possible that this pigment combination relates to zeaxanthin from small coccoid cyanobacteria species, that were not counted by the inverted microscopic analysis. Zeaxanthin+lutein, when measured, was higher at station 1 on 6/4/01 and 27/11/01, two periods of intense rainfall (see Fig. 3.27) and therefore considerable freshwater influence, suggesting that this pigment might be linked to the freshwater runoff. Cyanobacteria containing zeaxanthin may have been flushed into the Abbotsbury embayment, or if lutein, this may be related to vascular plant detritus, that would enter the lagoon through river discharge and runoff.

Violaxanthin is present in chlorophytes, prasinophytes and eustigmatophytes, but was only detected during 2001 in very low amounts (< 0.10 μg L^{-1}).

Particularly high phaeopigment concentrations were observed during the cryptophyte bloom and also during the *P. micans* bloom in 2000. Higher levels of phaeophorbides 1 and 2 relative to phaeophytin 1 and 2 were observed during these periods. Phaeophorbide molecules appear to be the most abundant of the phaeopigments in marine waters, and zooplankton grazing is considered to be the major pathway for phaeopigment production (Barlow et al. 1993b). These compounds appear to be a reliable indicator of physiological stress in both natural and laboratory cultures of phytoplankton

assemblages (Millie et al. 1993). In the Fleet lagoon phaeopigments were quite abundant from 5/9/00 to 3/10/00 at station 1, during the *P. micans* bloom period. Phaeopigments peaked on 3/10/00, indicating the senescence of the *P. micans* population and/or an increased grazing pressure, as a dramatic decrease in *P. micans* biomass was observed concurrent with the increase in phaeopigment concentrations at station 1. Increased abundances of the heterotrophic dinoflagellate *Oxyrrhis marina* were observed together with high abundances of *P. micans* at stations 1 to 5, which may indicate that *O. marina* was grazing on *P. micans*. Other microzooplankton species observed during the *P. micans* bloom were *M. rubra* and *Strombidium* sp. 1, but it is likely that macrozooplankton were also contributing to the grazing pressure upon *P. micans*, as microzooplankton abundance was not very high. Little is known about the zooplankton community within the lagoon, except that mysids are reported to be common (Dyrynda, 1997) and indeed high densities of the mysids *Neomysis integer* were observed in a net-trawl sample at Abbotsbury on 19/9/00.

On 1/6/00, presumably during the decline of the cryptophyte bloom (as higher chl-a was measured at station 1 in April 2001, indicating that the bloom might have started earlier in 2000) lower relative phaeopigment concentrations were observed and phaeophorbide 2 and phaeophytin 1 were the most abundant compounds among the phaeopigments on 1/6/00, while phaeophytin 2 peaked later on 14/6/00. Phaeophorbide 1 was not abundant in June 2000. Barlow et al. (1993b) reported that overall it appears that the temporal variation in phaeopigment distribution is a sensitive indicator of zooplankton grazing in response to changes in the structure of the phytoplankton community. On 1/6/00 a number of microzooplankton species were abundant, like the ciliates *M. rubra*, which has been shown to graze on cryptophytes, and *Strombidium* spp. and the heterotrophic dinoflagellate *C.* cf. *cohnii*. These species showed a marked decrease in abundance from 1/6/00 to 14/6/00, that might have been a response to the decreased prey availability on the latter date.

During 2001 phaeopigment concentrations were lower than 1 $\mu g\ L^{-1}$ during most part of the year, except for 27/11/01. Since phaeophorbides are formed by zooplankton grazing, the low concentration throughout the year suggests that zooplankton grazing was minimal during 2001. On 27/11/01, phaeopigment concentrations were higher, particularly of phaeophorbide 2 and phaeophytin 2 at stations 1, 3 and 4. On this date, chl-a was also high relative to the levels observed in late summer and autumn 2001, and microzooplankton grazers like the ciliates *M. rubra* and *Strombidium* spp. were found at these stations, although phytoplankton abundances were low. Another characteristic of

chromatograms from stations 1, 3 and 4 on 27/11/01 was the presence of increased levels of fucoxanthin, chl-b, zeaxanthin, β-carotene, 19' hexa (at stations 3 and 4) and violaxanthin, as well as phaeopigments. This was a very windy day, leading to resuspended bottom sediments. This suggests that the mixing conditions caused resuspension of benthic microalgae communities, that would account for the distinct pigment composition of these samples, as well as the presence of chl-a degradation products, that were associated with the detritus and bottom sediments of the lagoon.

4.5 Chapter summary

Overall, the pigment composition was a good descriptor of changes observed in the phytoplankton community. Blooms of cryptophytes in spring 2000 and 2001 and a dinoflagellate bloom in late summer-autumn 2000 could be identified, as well as the minor contribution of diatoms to the total phytoplanktonic biomass and on a few occasions dominated by chl-b containing organisms (euglenophytes). However, microscopy revealed changes in cell abundance and group specific species composition, which is highly valuable information for ecological research that can not be obtained by pigment analysis. This latter technique proved to be particularly useful to assess the taxonomic composition of nanoflagellates that could not be identified by microscopic analysis, as their taxonomy is poorly known, and their trophic status can not be determined using ordinary light microscopy. Therefore, chemotaxonomic identification may be a more powerful tool to identify these smaller algae, even when pigment interpretation is also difficult (Garibotti et al. 2003).

Some inconsistencies between the two techniques were observed however, as on two occasions when the presence of peridinin and fucoxanthin were not correlated with significant abundances of dinoflagellates and diatoms. Moreover, high biomass of *E. marina* was recorded by microscopic analysis at station 1 on 1/6/00, but no associated chl-b was detected by HPLC.

Therefore, HPLC based pigment analysis can be considered complementary to, but not exclusively a replacement for microscopic enumeration (Tester et al. 1995, Henriksen et al. 2002, Garibotti et al. 2003). As stated by Millie et al. (1993), pigment analysis coupled with microscopic enumeration would provide the most accurate characterization of algal assemblages.

Chapter 5

Toxic *Alexandrium minutum* from the Fleet lagoon

5. 1 Introduction

The species *Alexandrium minutum* was first described in 1960 from Alexandria Harbour in Egypt (Halim, 1960). Since then, this species has been reported to occur in coastal waters of Portugal, Spain, Italy, France, Taiwan, Australia, New Zealand (Taylor et al. 1995b) and Peninsula Malaysia (Usup et al. 2002). *A. minutum* blooms occur in enclosed areas of harbours and lagoons, and in estuaries and are usually associated with stable water column conditions and enhanced freshwater inputs (Delgado et al. 1990, Giaccobbe et al. 1996). Moreover, it is hypothesized that germination of cysts from bottom sediments may play an important role as an inoculum for *A. minutum* blooms, as described for other species of *Alexandrium* (Anderson, 1998). Cysts of *A. minutum* were first described in sediments from Port river, in south Australia (Bolch et al. 1991) and they have also been found in sediments from the Bay of Morlaix, in France, where *A. minutum* is among the most frequently encountered planktonic dinoflagellate species.

A. minutum was first found to produce PSP toxins in 1988 (Hallegraeff et al. 1988). PSP toxins include a family of highly potent neurotoxins with more than 20 naturally occurring analogues, including carbamate, N-sulfocarbamoyl, decarbamoyl and deoxy-decarbamoyl derivatives (Cembela, 1998). These toxins can be accumulated by shellfish and transmitted through the food chain causing the PSP syndrome in humans. *A. minutum* has been responsible for PSP contamination of shellfish in aquaculture areas where it blooms, including Port river in Australia (Oshima et al., 1989), Brittany in France (Belin, 1993), the Galician rias of Spain (Franco et al. 1994) and the Bay of Plenty in New Zealand (Chang et al. 1997).

The profile of PSP toxins produced by *A. minutum* varies in different geographic areas, but in general is dominated by the highly potent carbamate toxins (GTX-1,-2,-3,-4, STX and NeoSTX), primarily GTX's, except for some New Zealand isolates. The less potent n-sulfocarbamoyl toxins, C1 and C2 were found in only one strain from France (Chang et al. 1997) while dc-GTX-2 and dc-GTX3 were found in this same French strain by Bechemin et al. (1999). Dc-STX was found as a minor constituent of the PSP profile of strain AL2V isolated from Ria de Vigo, Spain (Carreto et al. 2001) and deoxy-decarbamoyl derivatives were not found in this species.

In the UK, PSP has been found in shellfish from the northeast coast nearly every year since 1968. More recently, since 1996, when the monitoring program was expanded to include the south coast and Wales, shellfish containing PSP toxins have also been found in the Fal and Fowey estuaries on the southwest coast and in Milford Haven harbour, in south Wales (Higman and Morris, 2000). The organism responsible for incidents of PSP on the northeast coast has been reported to be *Alexandrium tamarense* (Robinson, 1968) and it has been generally assumed that this same species is responsible for PSP incidents that occur in other areas of the UK coast. However, *A. tamarense* strains isolated from the Tamar estuary, Plymouth and from the Weymouth inner harbour, both on the south coast, have been shown to be non toxic (Cembella et al. 1987, Higman et al. 2001).

Blooms of species identified as *A. tamarense* and *Alexandrium* sp. were recorded in the Fleet lagoon in 1996 and 1998 (Jamieson, 1998), but no associated PSP toxins were detected in locally cultivated oysters or mussels. *Alexandrium minutum* has not been previously recorded in the Fleet lagoon.

In this chapter results of a growth experiment are reported, when toxin concentrations, pigment composition, growth rate and nitrate and phosphate consumption from the growth media by *A. minutum* from the Fleet lagoon were determined during its growth in batch culture.

5.2 Results

5.2.1 Species morphology and identification

Cells of *A. minutum* strain 3.9h are ovoid to round, measuring 23 ± 3 μm long and 21 ± 3 μm wide (Fig. 5.1). The dinoflagellate was only observed as single cells in culture. The 6'' plate is narrow, and the posterior sulcal plate is wider than long in agreement with previous observations of this species (e.g., Balech, 1995) (Fig. 5.1). The apical pore complex (APC) is narrowly triangular with no attachment pore. The cingulum is deeply excavated and is displaced 1 girdle width.

The presence of a ventral pore on the suture between the first and fourth apical plates has been used to distinguish the species (Taylor et al. 1995), but this feature was variable in this strain (Fig. 5.1).

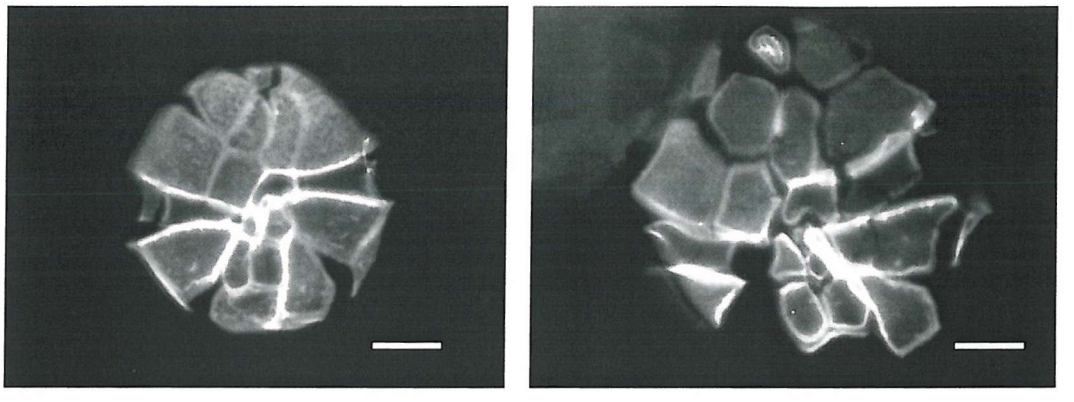


Figure 5.1: Light micrographs of *A. minutum* strain 3.9h stained with calcofluor. Scale bar = 5 μm.

5.2.2 Growth rate

A. minutum strain 3.9h was grown at 17 °C, 120 μmoles photon flux m^{-2} s^{-1}, and a light:dark cycle of 12 h:12 h. The exponential growth phase lasted 9 days and a maximum mean cell density of 17809 cells mL^{-1} was attained. Although samples were only collected on days 1, 5 and 9 during the exponential growth phase, the estimated growth rate was of 0.25 day^{-1} (Fig. 5.2).

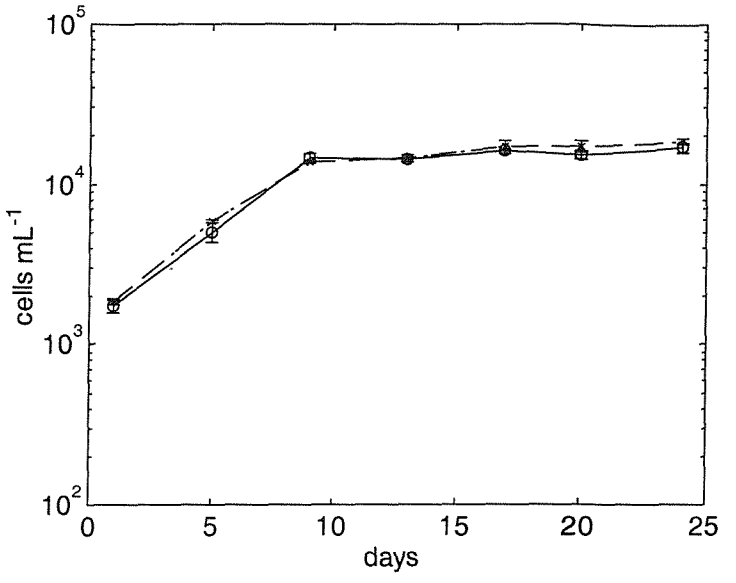


Figure 5.2: *Alexandrium minutum* growth experiment showing changes in cell numbers. Continuous line with open circles corresponds to culture 1 and dashed line with x to culture 2. Each point is a mean of two measurements, error bars corresponds to ± 1 standard difference.

5.2.3 Nitrate and phosphate consumption from growth media

Nitrate concentrations in the growth media decreased from 888 μM on day 1 to around 650 μM on day 13 and phosphate concentrations that were initially 38.8 μM, diminished to 6.0 μM on day 20 (Fig. 5.3). Between days 5 to 9 the consumption of nitrate was on average 23.9 μM day^{-1}, decreasing to 0.5 μM day^{-1} from day 20 to 24, although nitrate concentrations in the media remained as high as 615 μM (Table 5.1).

Table 5.1: Mean nitrate and phosphate consumption from growth media (μM day^{-1}) by *A. minutum* 3.9h. Numbers in brackets represent standard difference of two measurements.

Time range (days)	Nitrate	Phosphate	N:P ratio
1-5	15.5 (2.0)	1.3 (0.9)	11.9
5-9	23.9 (8.2)	2.8 (0.4)	8.5
9-13	20.2 (2.7)	3.4 (0.0)	5.9
13-17	4.5 (1.9)	0.8 (0.1)	5.6
17-20	4.9 (0.9)	0.0 (0.1)	-
20-24	0.5 (1.2)	-	-

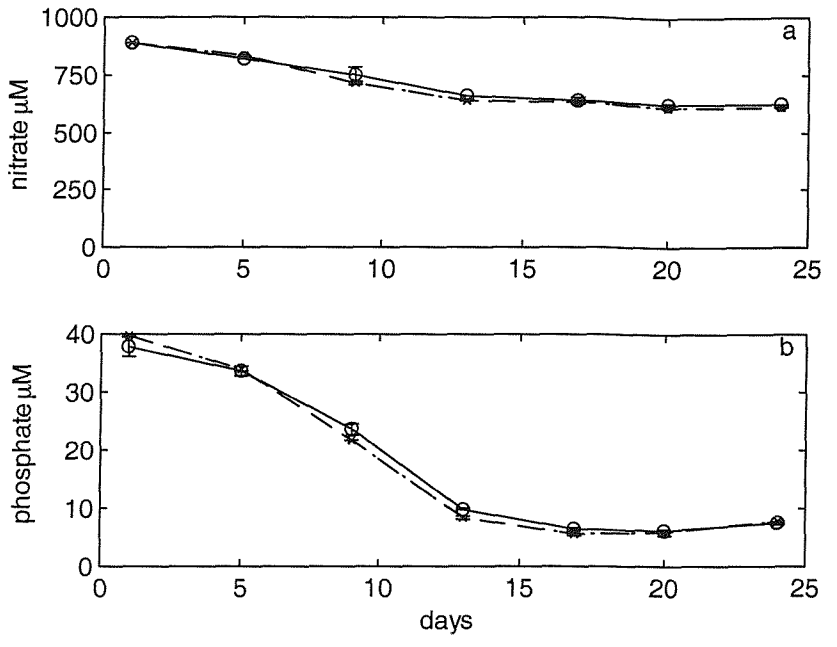


Figure 5.3: *Alexandrium minutum* growth experiment showing changes in a) nitrate and b) phosphate concentrations in the growth media. Open circles correspond to culture 1 and x to culture 2. Each point is a mean of two measurements and error bars corresponds to ± 1 standard difference.

From day 9 to 13, phosphate consumption from the growth media was on average 3.4 μM day^{-1}, and decreased to 0.0 μM day^{-1} from day 17 to 20 (Table 5.1). An increase in phosphate concentration was observed from day 20 to 24 and was probably caused by cell lysis or phosphate excretion, as the culture was in late stationary phase. Nitrogen and phosphate were not exhausted from the culture media, although cells ceased growth around day 13 with residual nitrate and phosphate levels of 615 μM and 7.5 μM resulting on day 24. The nitrate to phosphate draw-down ratio, calculated from the slope of the linear regression of nitrate and phosphate concentrations in the growth media from day 1 to day 20 was 7.8 (Fig. 5.4). This value is well below the N:P Redfield ratio of 16:1 for particulate matter in the sea. However, Geider and La Roche (2002) have shown that inorganic N:P draw-down ratios can range from 4.4 to 19 and that values lower then 16:1 are associated with nutrient replete-cells with an intracellular accumulation of phosphate.

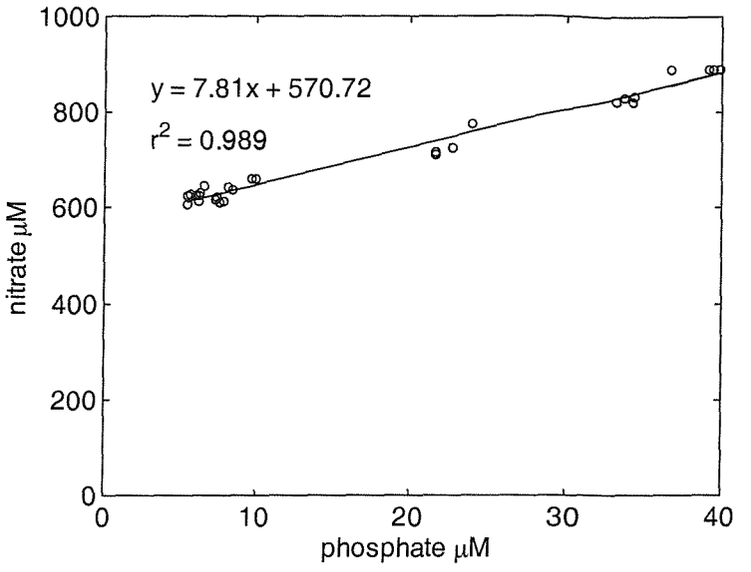


Figure 5.4: Nitrate against phosphate concentrations in the growth media, showing the equation of the line and correlation coefficient (n = 28, $p < 0.01$). Two replicas for each of two culture flasks on every sampling date were included (four points per date).

5.2.4 Pigment composition

Chlorophyll-a values determined fluorometrically (Fig. 5.5) and by HPLC (Fig. 5.6a) were compared and a good agreement was observed between them, as shown by the values of the regression correlation coefficient, $r^2 = 0.98$ (Fig. 5.7), although the slope of the line derived from the data plot was greater than 1.

HPLC analysis has shown that strain 3.9h contains chlorophyll-a, peridinin and diadinoxanthin as major pigments, and chlorophyll-$c_{1+}c_2$ (not separated by the present HPLC method) and c_3, diatoxanthin, and β-carotene as minor components (less than 1 pg $cell^{-1}$). A peak with the retention time of 19'- butanoyloxyfucoxanthin was also observed, but its identity could not be confirmed by absorption spectrum analysis due to the small concentration. No peak with an absorption spectrum similar to dinoxanthin was observed from this isolate.

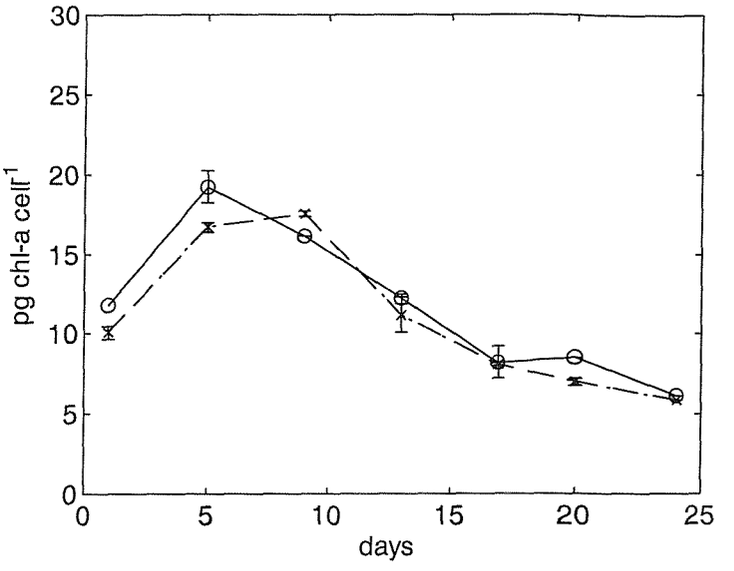


Figure 5.5: *Alexandrium minutum* growth experiment showing changes in chl-a concentration per cell (measured fluorimetrically). Continuous line with open circles corresponds to culture 1 and dashed line with x to culture 2. Each point is a mean of two measurements, error bars corresponds to ± 1 standard difference.

The chlorophyll-a content per cell increased from 9.0 pg cell^{-1} on day 1 to a peak during the exponential growth phase of 16.0 pg chl-a cell^{-1} on day 5. Chlorophyll-a concentrations decreased rapidly after day 13, when nitrate consumption ceased (Fig. 5.3), attaining a minimum value of 5.4 pg chl-a cell^{-1} on day 24 (Fig. 5.6a). The amounts of peridinin, diadinoxanthin and β-carotene per cell covaried with that of chlorophyll-a per cell (Fig. 5.6), however, the concentration of chlorophyll-$c_{1+}c_2$ showed a different trend, increasing gradually from day 1 to 13, then decreasing to almost zero on day 17 (Fig 5.6f). Diatoxanthin concentrations showed the tendency of a gradual increase throughout the growth curve (Fig. 5.6d). Chlorophyll-a/peridinin ratios of 2.6 were observed on day 1, while on day 5 this ratio had decreased to 1.8, then gradually further declining to 1.3 on day 24 (Fig. 5.8a). The relative proportion of chlorophyll-a/diadinoxanthin showed higher values, of 8.2 on day 1, that declined to 5.4 on day 5 and then gradually decreased to 4.5 on day 24 (Fig. 5.8b).

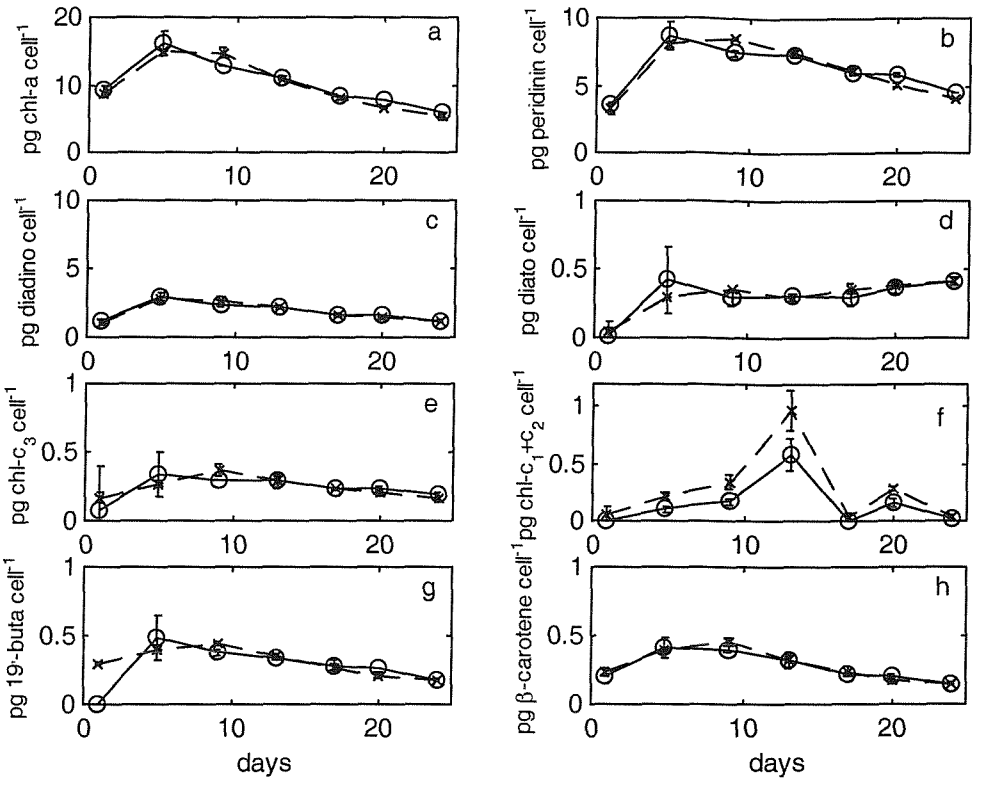


Figure 5.6: Changes in pigment contents of *A. minutum* (measured by HPLC); (a) chlorophyll-a, (b) peridinin, (c) diadinoxanthin, (d) diatoxanthin, (e) chlorophyll-c_3, (f) chlorophyll-c_1+c_2, and (g) 19'buta, and (h) β-carotene. Continuous line with open circles corresponds to culture 1 and dashed line with x to culture 2. Each point is a mean of two measurements and error bars corresponds to ± 1 standard difference.

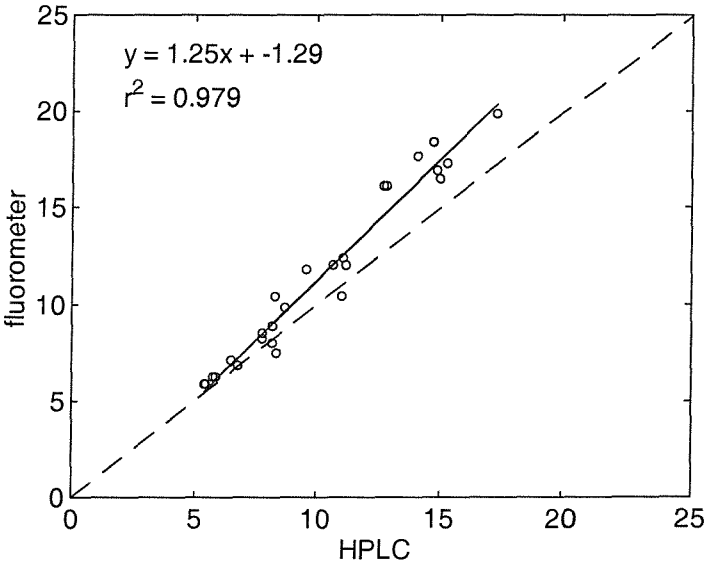


Figure 5.7: Chl-a values measured by fluorescence against chl-a values measured by HPLC (pg chl-a $cell^{-1}$). The solid line represents a linear regression fit to the data and the equation and correlation coefficient for this line are shown (n = 27, $p < 0.01$). Two replicas for each of two culture flasks on every sampling date were included (four points per date). The dashed line represents the 1:1 agreement line.

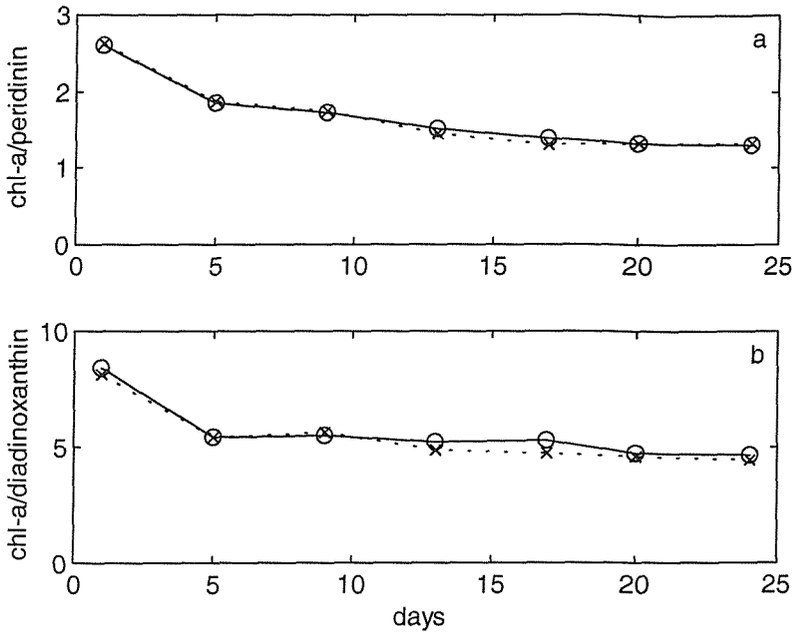


Figure 5.8: a) chl-a to peridinin ratio, b) chl-a to diadinoxanthin ratio. Continuous line with open circles corresponds to culture 1 and dashed line with x to culture 2.

5.2.5 PSP toxins

The total toxin content per cell varied from 5.61 fmol $cell^{-1}$ on day 1 to a maximum of 16.84 fmol $cell^{-1}$ during early stationary phase (day 13), and was quite constant until day 20, decreasing slightly on day 24 (Fig. 5.9a, Table 5.2). Total toxicity per volume of media (pmol toxin mL^{-1}) showed the same trend as toxicity per cell, peaking at 244.32 pmol mL^{-1} on day 13 (Fig. 5.9b). When total toxicity was calculated on a chlorophyll-a basis (measured by fluorometry, used here as this is the method more generally employed to determine chlorophyll-a concentrations), a gradual and constant increase was detected towards late stationary phase (Fig. 5.9c), due to the decrease in chlorophyll-a per cell, observed from day 9 to day 24 (Fig. 5.5).

Toxin composition profile showed the presence of GTX-3, GTX-2 and STX (Fig. 5.10a, b and 5.11, Table 5.2). Neither NeoSTX or GTX-1 and -4, were detected in this isolate. The presence of STX was confirmed by LC/MS analysis, as shown in Fig. 5.12. GTX-3 was the dominant toxin constituent, followed by STX, while GTX-2 was a minor component. The contribution of each toxin (mole %), in late exponential phase was 54.3 % GTX-3, 9.6 % GTX-2 and 36.1 % STX (Table 5.2). The toxin profile of this isolate only changed slightly during the stationary growth phase (Table 5.2, Fig. 5.10a). The GTX-3 contribution (mole %) gradually decreased from 54.3 % in late exponential phase (day 9) to 37.5 % in late stationary phase (day 24), while STX increased from 36.1 % to

45.4 % during this period. The contribution of GTX-2 remained quite constant at around 14 %, increasing to 17 % on day 24.

Table 5.2: Average concentrations of STX, GTX-3 and GTX-2 of *A. minutum* cell pellets during a growth experiment in a batch culture. Numbers in parentheses are standard deviation, n = 4.

	Day of culture	GTX-3	GTX-2	STX	TOTAL
Average pg cell^{-1} (S.D.)	1	1.16 (0.36)	0.26 (0.08)	0.61 (0.32)	2.0
	9	3.43 (0.22)	0.61 (0.02)	1.73 (0.10)	5.8
	13	3.25 (0.15)	0.80 (0.05)	1.98 (0.10)	6.0
	17	2.53 (0.20)	0.78 (0.04)	1.76 (0.13)	5.1
	20	2.42 (0.15)	0.86 (0.03)	1.96 (0.08)	5.2
	24	1.75 (0.14)	0.79 (0.07)	1.61 (0.14)	4.1
Average fmol cell^{-1} (S.D.)	1	2.93 (0.04)	0.67 (0.06)	2.02 (0.31)	5.6
	9	8.67 (0.38)	1.53 (0.01)	5.76 (0.21)	16.0
	13	8.22 (0.10)	2.03 (0.04)	6.59 (0.04)	16.8
	17	6.38 (0.53)	1.98 (0.09)	5.85 (0.44)	14.2
	20	6.12 (0.16)	2.17 (0.03)	6.51 (0.01)	14.8
	24	4.41 (0.40)	2.01 (0.19)	5.34 (0.50)	11.8
Average mole% (S.D.)	1	53.9 (6.4)	12.5 (3.0)	33.6 (9.3)	-
	9	54.3 (0.3)	9.6 (0.3)	36.1 (0.1)	-
	13	48.8 (0.5)	12.0 (0.3)	39.2 (0.3)	-
	17	44.9 (0.3)	14.0 (0.3)	41.2 (0.1)	-
	20	41.3 (0.7)	14.7 (0.3)	44.0 (0.6)	-
	24	37.5 (0.2)	17.1 (0.1)	45.4 (0.1)	-
Average pg STXequiv cell^{-1} (S.D.)	1	0.88 (0.27)	0.13 (0.04)	0.61 (0.32)	1.6 (0.62)
	9	2.61 (0.17)	0.29 (0.01)	1.73 (0.10)	4.6 (0.28)
	13	2.47 (0.11)	0.39 (0.02)	1.98 (0.10)	4.8 (0.23)
	17	1.92 (0.15)	0.38 (0.02)	1.76 (0.13)	4.1 (0.30)
	20	1.84 (0.11)	0.41 (0.01)	1.96 (0.08)	4.2 (0.20)
	24	1.33 (0.11)	0.38 (0.03)	1.61 (0.14)	3.3 (0.28)

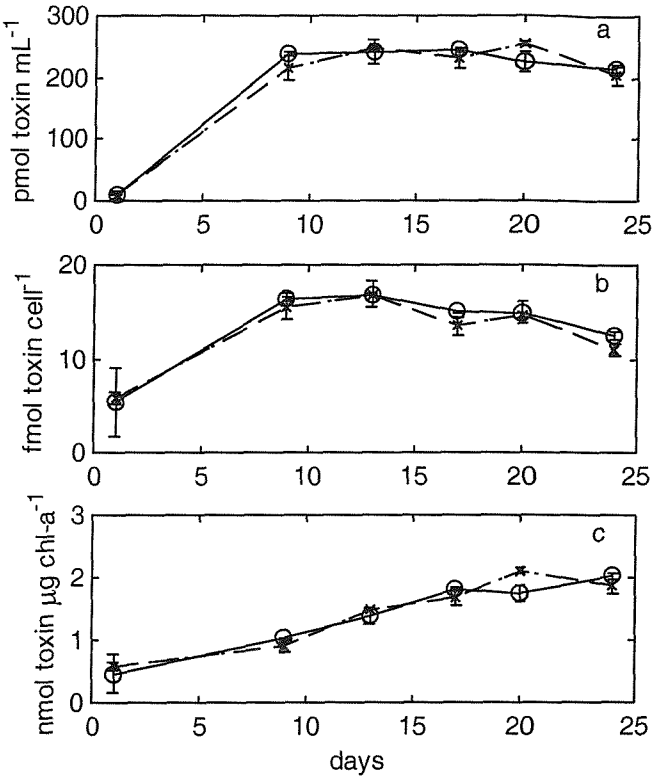


Figure 5.9: *Alexandrium minutum* growth experiment showing changes in total toxin content (a) per culture volume, (b) toxin per cell and (c) toxin per unit of chl-a. Continuous line with open circles corresponds to culture 1 and dashed line with x to culture 2. Each point is a mean of two measurements, error bars corresponds to ± 1 standard difference.

Maximum concentration of GTX-3 of 8.7 fmol cell^{-1}, was attained in late exponential growth phase, while maxima of STX (6.6 fmol cell^{-1}) and GTX-2 (2.2 fmol cell^{-1}) were observed during the stationary phase (Table 5.2, Fig. 5.10b). When the concentration of each toxin was converted to STX equivalents to provide a measure of the potency of the strain, the average toxicity of the culture was 3.8 pg STXequiv cell^{-1}, varying from 1.6 on day 1 to a maximum of 4.8 pg STXequiv cell^{-1} during early stationary phase (Table 5.2, Fig. 5.10c).

PSP toxins were not detected in oysters and cockles collected from the Fleet lagoon during 2001.

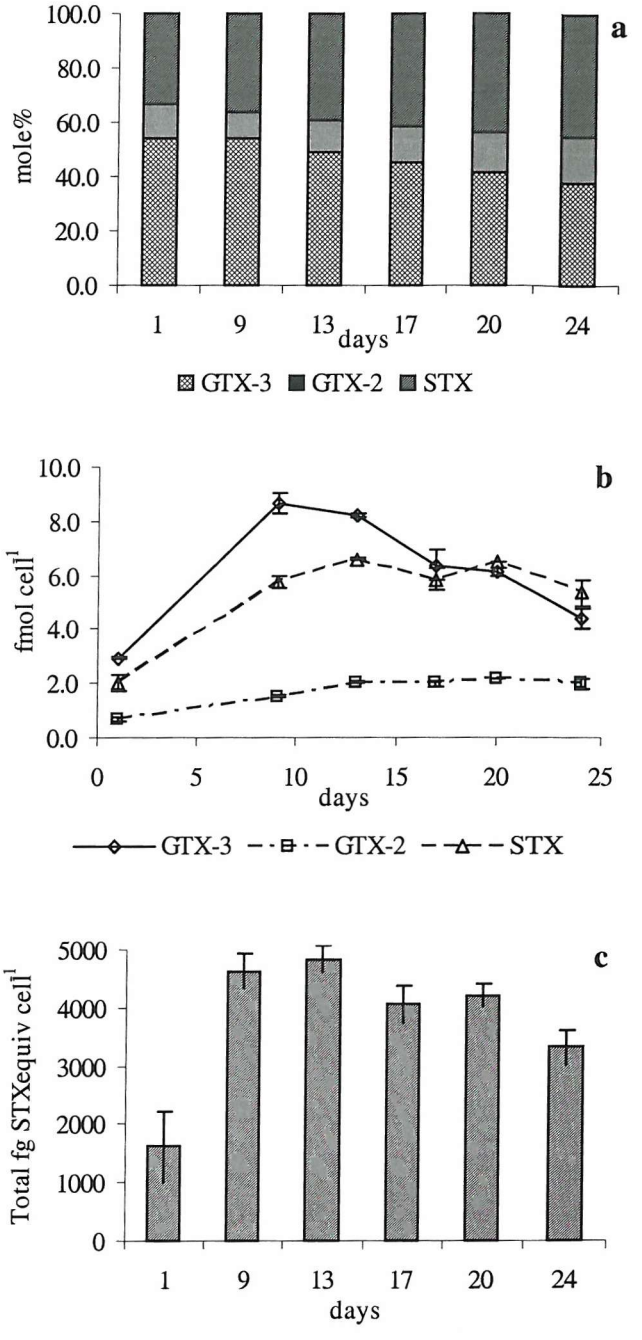


Figure 5.10: *Alexandrium minutum* growth experiment showing changes in (a) mole % of GTX-2, GXT-3 and STX, (b) GTX-2, GXT-3 and STX concentrations (fmol cell^{-1}) and (c) cellular potency (fg STXeq cell^{-1}). Each value corresponds to a mean of four measurements and error bars corresponds to ± 1 standard deviation.

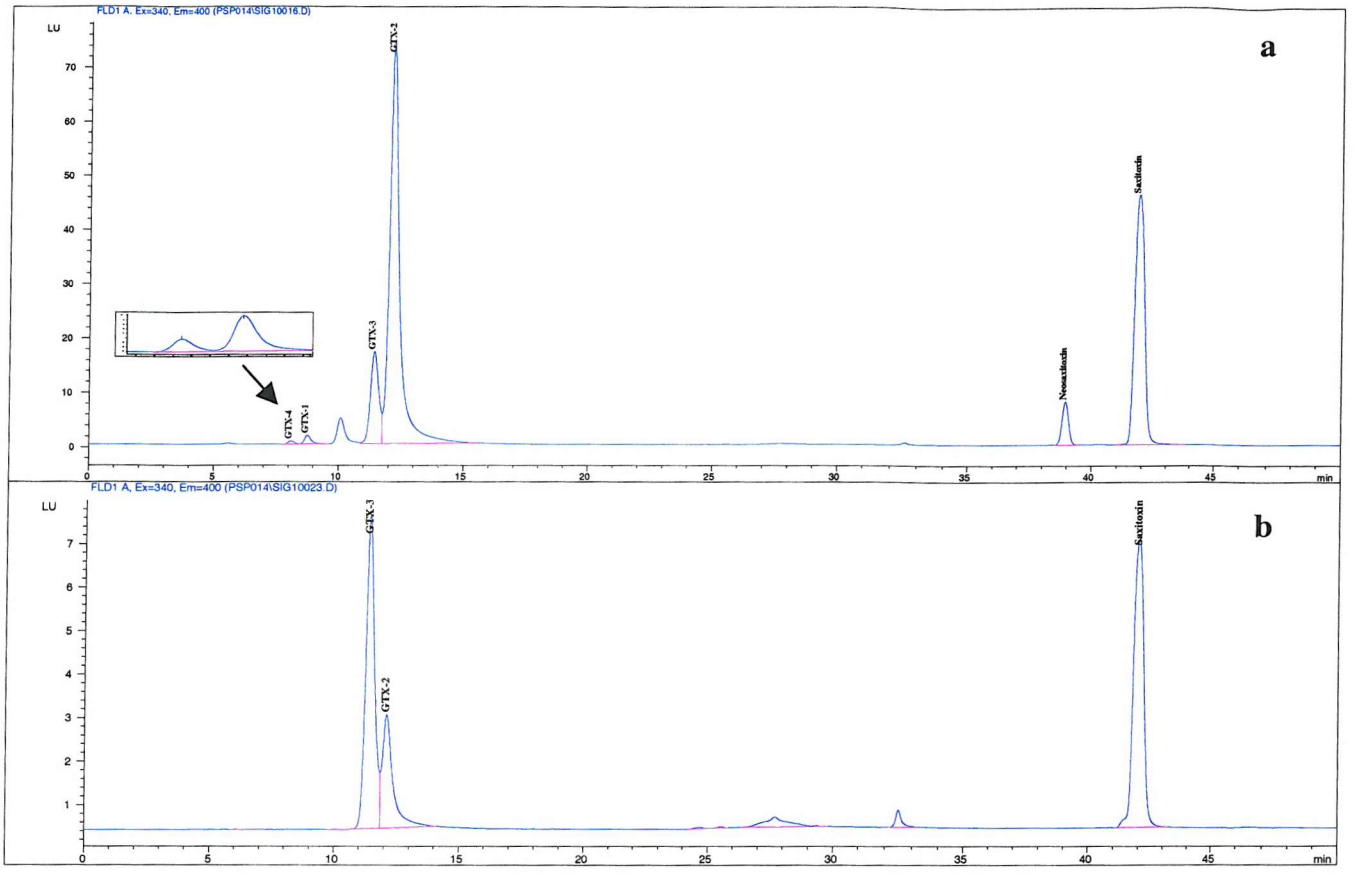


Figure 5.11: HPLC chromatograms. (a) Toxin calibration standard mixture (GTX-1,-2,-3,-4, STX and NeoSTX), insert shows GTX-1 and -4 peaks magnified, (b) *A. minutum* 3.9h extract (day 20 growth experiment).

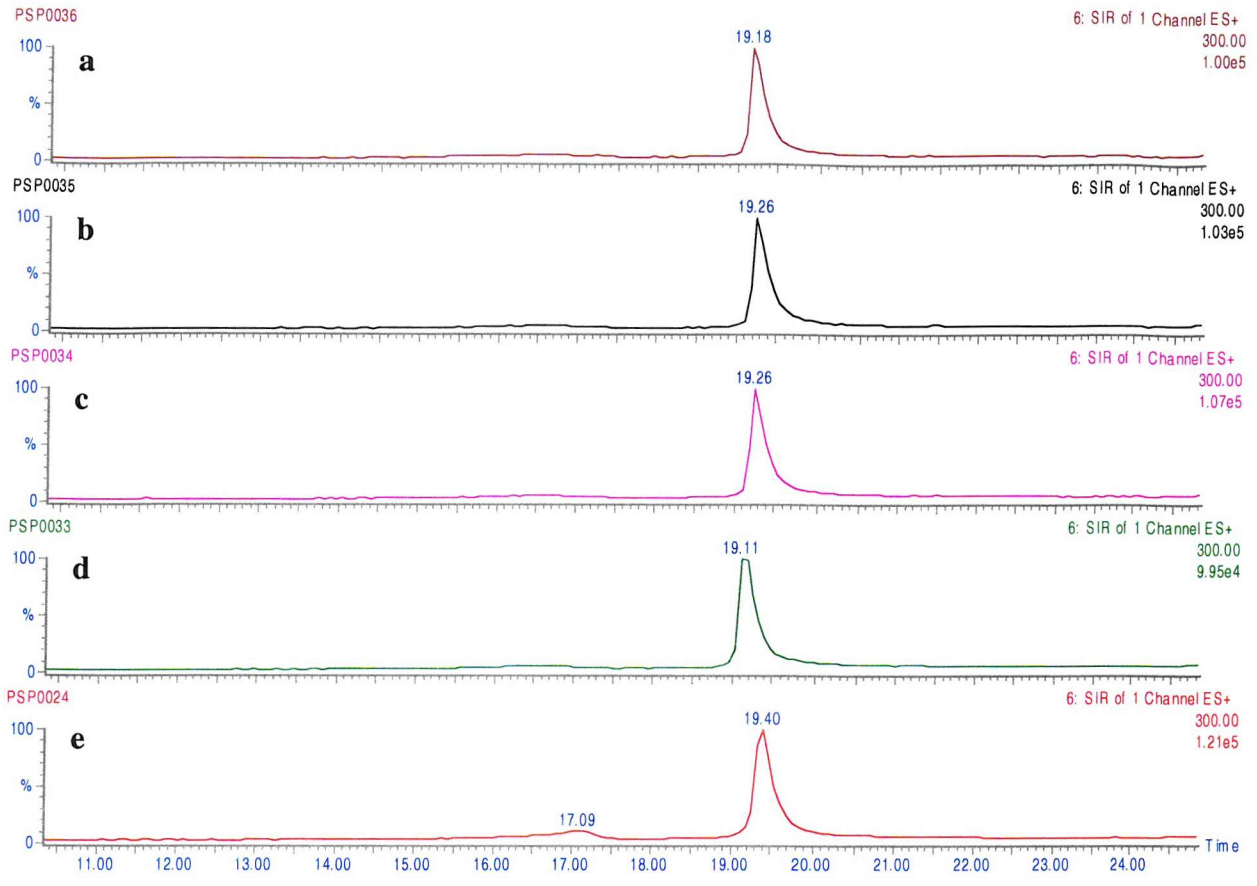


Figure 5.12: Chromatographic elution of saxitoxin (STX) using selected ion monitoring m/z 300 $[M+H]^+$. a-d Chromatograms are selected replicate extracts of the *A. minutum* 3.9h from (a) day 24, (b) day 20, (c) day 13, (d) day 9 and (e) saxitoxin reference standard.

5. 3 Discussion

A. minutum 3.9h ceased growth around day 13 in batch culture, although concentrations of nitrate and phosphate in the growth media were 615 μM and 6.0 μM. Dinoflagellates often cease growth when there are still excess amounts of macronutrients present in the medium (Flynn et al. 1994), and this may be due to dissolved inorganic carbon stress, especially under high cell densities (Anderson et al. 1990, Flynn et al. 1996).

It is of interest that peridinin, diadinoxanthin and β-carotene covaried with chlorophyll-a. Peridinin and diadinoxanthin are light harvesting pigments in dinoflagellates, as is chlorophyll-c_2, that was expected to covary with chlorophyll-a. However, chlorophyll-c_1+c_2 revealed a distinct temporal pattern, peaking on day 13, when all the other pigments had started to decline. β-carotene, diadinoxanthin and diatoxanthin are suggested to have a photoprotective role, the later two via the xanthophyll cycle.

Demers et al. (1991) observed a fast and reversible increase in the concentration of the epoxy-free diatoxanthin, at the expense of its parent compound, diadinoxanthin, in *Alexandrium excavatum* upon exposure to high light. Data presented in Fig 5.6 suggest that a small fraction of the diadinoxanthin pool, that decreased from 2.8 pg $cell^{-1}$ on day 5 to 1.2 pg $cell^{-1}$ on day 24, might have been converted to diatoxanthin, that increased from 0.03 pg $cell^{-1}$ on day 1 to 0.41 pg $cell^{-1}$ on day 24. Interestingly, diatoxanthin was not detected in two other strains of *A. minutum* (AL1V and AL2V) studied by Flynn et al. (1994), and the authors argued this could be due to the "low" photon flux densities of 200 μmol $m^{-2}s^{-1}$ employed in their experiments. Cultures in the current study were grown at irradiances of 120 μmol $m^{-2}s^{-1}$, and diatoxanthin was observed at a maximum concentration of 0.4 pg $cell^{-1}$. Indeed, Carreto et al. (2001) reported the presence of trace amounts of diatoxanthin in shade adapted cells of strain AL2V, although they do not give details of growth irradiance levels.

The relative proportions of chlorophyll-a/peridinin and chlorophyll-a/diadinoxanthin on day 1 of the experiment were higher than the ones reported by Flynn et al. (1994) for two strains of *A. minutum* growing at 200 μmol $m^{-2}s^{-1}$, while values from day 5 to day 24 were more comparable.

The HPLC toxin analyses of *A. minutum* strain 3.9h have revealed the presence of STX, GTX-2 and GTX-3. The toxin composition of *A. minutum* 3.9h is quite different to that reported for other isolates of this species from sites throughout the world. This strain has a unique toxin profile containing GTX-2 (9.6 mole %), GTX-3 (54.3 mole %) and STX (36.1 mole %). Strains of *A. minutum* isolated from the Fal estuary, on the southwest UK peninsular have been found to produce only GTX-2 (23.5 mole %) and GTX-3 (76.5 mole %) toxins (Percy et al. 2002). A French strain isolated from Morlay Bay in Brittany, showed a mole % of GTX-2 and GTX-3 (56 mole %) similar to strain 3.9h, but contains C1 and C2 toxins instead of STX (Chang et al. 1997). Other *A. minutum* strains isolated from Spain (Franco et al. 1994), Portugal (Cembella et al. 1987) and Taiwan (Hwang and Lu, 2000) produced only gonyautoxins, primarily GTX-1 and -4 (64 to 98 mole %). However, Carreto et al. (2001) observed small amounts of GTX-2, GTX-3, C1, C2, NeoSTX and dcSTX in addition to the major toxins, GTX-1 and GTX-4 in one of the strains studied by Franco et al. (1994). Differences in growth conditions were cited to account for the discrepancies observed.

Australian strains also produce only gonyautoxins, predominantly GTX-1 and -4 (73.5 and 82.9 mole %), and considerable amounts of GTX-2 and -3 (26.5 and 17.1 mole %) (Hallegraeff et al. 1991). *A. minutum* isolates from New Zealand (Chang et al. 1997,

MacKenzie and Berkett, 1997) have a more complex toxin profile, containing GTX-1 and -4 (7.6 to 96.4 mole %), GTX-2 and -3 (2.9 to 36 mole %) STX (0.7 to 31 mole %) and NeoSTX (0.0 to 77.2 mole %), showing highly variable toxin profiles among different isolates. *A. minutum* strains isolated from a coastal lagoon on the Peninsula Malaysia have shown to produce GTX-1 and -4 and NeoSTX (Usup et al. 2002).

It is clear that the toxin profile of the Fleet lagoon isolate of *A. minutum* does not resemble any other isolate characterized to date. The production of STX is unique among the European *A. minutum* isolates, and the predominance of GTX-3 and STX is unusual among global populations. Furthermore, this profile is also distinct from the only other *A. minutum* strains reported from the UK, that produce only GTX-2 and GTX-3 (Percy et al. 2002). Interestingly, strains from the south coast of England and from Brittany in France, on the other side of the English Channel, produce predominantly GTX-3 and GTX-2, in contrast to the dominance of GTX-1 and GTX-4 in the toxin profile of other European isolates from the Iberian Peninsula.

Considering the cellular potency of the isolate (maximum of 4.8 pg STX equiv $cell^{-1}$), strain 3.9h is comparable to one isolate from Spain (6.4 pg STXequiv $cell^{-1}$) and one from New Zealand (6.0 pg STXequiv $cell^{-1}$) (Chang et al. 1997). *A. minutum* isolates from the Fal estuary have shown to be less toxic, producing 1.5 pg STXequiv $cell^{-1}$ (Percy et al. 2002). In general, there is considerable variability among different isolates, according to the toxin profile and the relative abundance of each compound, with strains from Portugal and France the least potent, while two strains from Taiwan are the most toxic (20.6 and 47.0 pg STXequiv $cell^{-1}$). The potency of the New Zealand strains ranges from 0.88 to 11.59 pg STXequiv $cell^{-1}$ (Chang et al. 1997, MacKenzie and Berkett, 1997). Considerable differences were observed between strains from geographically close areas and therefore the analysis of a larger number of strains is desirable to allow a better picture of geographic toxicity variability in this species.

The predominant PSP toxin components throughout the growth curve of strain 3.9h were GTX-3 and STX. Maximum cellular toxicity was attained during early stationary phase and after this point toxicity began to decrease. A similar trend in toxicity was observed in a Taiwan strain (Hwang and Lu, 2000). Flynn et al. (1994) reported maximum toxicity of *A. minutum* when cells were recovering from lag phase and nitrogen deprivation, suggesting a link between an upshock in nitrogen status of the cells and toxin synthesis. *A. minutum* 3.9h cells were already in exponential phase at the start of the growth experiment and did not appear to be nitrogen limited.

Strains of other *Alexandrium* species from the south coast of England, such as *A. tamarense* isolated from Plymouth (Cembella et al. 1987), from Weymouth Harbour (Higman et al. 2001b) and from the Fal estuary (Lilly et al. 2002) are known to be non toxic. This suggests that the *A. tamarense* population from the southwest of England is non toxic, and might indicate that *A. minutum* is the source of PSP toxins found in shellfish in this region.

A. minutum had not been previously reported to occur in the Fleet and strain 3.9h was isolated from the lagoon in September 2000 during a bloom of *Prorocentrum micans* during which it was just a minor constituent of the phytoplankton community. It is well known that blooms of *A. minutum* occur in enclosed areas with freshwater input like shallow harbours, estuaries and coastal lagoons (Hallegraeff et al. 1988, Cannon, 1990, Delgado et al. 1990, Giacobbe et al. 1996). Although PSP toxins were not detected in oysters acquired from the Fleet, the enclosed nature of this lagoon, together with high nutrient inputs may favour blooms of *A. minutum* in the future, and these may become a threat to the local oyster farming. Indeed, a bloom of *A. minutum* that discoloured the waters of the Fleet lagoon was observed in July 2003 during the Environment Agency (EA) monitoring (R. Acornley, pers. comm.). The EA has been monitoring the Fleet lagoon since 1994 and blooms of *A. tamarense* and *Alexandrium* sp. have been recorded during some of these years (Jamieson, 1998). *A. tamarense* is known to reach high cell numbers in most years in Weymouth Inner Harbour, which connects with Portland Harbour close to the mouth of the Fleet lagoon. It is difficult to confirm that the first record of the occurrence of *A. minutum* in this region in September 2000 represents a new introduction, as it is possible that the species may have been misidentified in the past, given the morphological similarities within the *Alexandrium* genus (Balech, 1995). It might well be possible that *A. minutum* was part of a "hidden flora", and escaped detection for a long time due to low cell abundance. However, the transport of dinoflagellate cysts via ships ballast water or with shellfish seed stocks is documented in the literature (Hallegraeff and Bolch, 1991), and *A. minutum* cysts have already been found in sediments collected from ballast tanks of ships arriving in UK ports (Hamer et al. 2001).

The unique toxin profile of this isolate suggests a local origin. However, it is also possible that this strain was translocated from a population that has not been characterized yet, as the Fleet lagoon is connected to Portland Harbour, which was a busy naval port in the past, and the possibility of its anthropogenic introduction via ballast water remains open. The introduction of *A. catenella* from the Western Pacific to the coastal Mediterranean Thau lagoon in France was recently reported by Lilly et al. (2002). These

authors hypothesized that a ship released ballast water containing *A. catenella* cysts that originated from Asia, in the port of Sète, that communicates with the Thau lagoon. It is possible that a similar history has happened in Portland Harbour, leading to the introduction of *A. minutum* in the Fleet lagoon.

During the survey of dinoflagellate cysts in the surface sediments of the Fleet lagoon, low abundances (20 cysts per gram of wet sediment) of a small cyst with similar dimensions to *A. minutum* cysts were found in the innermost region of the lagoon. However, an exact identification requires the hatching and identification of the motile stage (see chapter 3).

5.4 Chapter summary

A. minutum strain 3.9h isolated from the Fleet lagoon presented an estimated growth rate of 0.25 day^{-1}. Pigments detected by HPLC were chlorophyll-a, peridinin and diadinoxanthin as major pigments, and chlorophyll-$c_{1+}c_2$, chl-c_3, diatoxanthin, and β-carotene as minor components. *A. minutum* 3.9h presented maximum nitrate and phosphate consumption of 24 and 2.8 μM day^{-1} during the exponential growth phase.

A. minutum 3.9h toxin profile consists of GTX-2 (9.6 mole %), GTX-3 (54.3 mole %) and STX (36.1 mole %), and is distinct from the toxin profile of any other isolate characterized to date. The predominant PSP components throughout the growth curve of strain 3.9h were GTX-3 and STX. Maximum cellular potency of the isolate was of 4.8 pg STX equiv $cell^{-1}$ during early stationary growth phase.

Blooms of *A. minutum* have increased in frequency and distribution in the estuaries of Brittany, northern France, since the first recorded occurrence in 1988 (Erard-Le Denn et al. 2000), inducing shellfish contamination by PSP. Considering that *A. minutum* may be the source of PSP toxins in the south coast of the UK, more studies about *A. minutum* abundances and the possible presence of cyst beds in sediments from this region are desirable to assess the risks of PSP contamination of shellfish caused by this species.

Chapter 6

Toxic *Prorocentrum lima* from the Fleet lagoon

6.1 - Introduction

Prorocentrum lima is an epiphytic-benthic dinoflagellate widely distributed in tropical and temperate coastal waters (Faust, 1991). The species occurs in sand (Dodge, 1982), attached to the surface of macroalgae and benthic debris, associated with coral reefs, or attached to floating detritus in a mangrove habitat (Faust, 1991). It is an important primary producer among the benthic dinoflagellate species (Carlson and Tindall, 1985). *P. lima* has been shown to produce the lipophylic polyether compounds, okadaic acid (OA), dinophysistoxin-1 (DTX-1) and DTX-2, the principal toxins responsible for diarrhetic shellfish poisoning (DSP) in humans (Murakami et al. 1982). *P. lima* has also been linked to the tropical syndrome ciguatera, as it is a frequent component of the epiphytic community of ciguatera-endemic areas (Tindall and Morton, 1998).

There are few studies recording *P. lima* abundance in temperate waters. Vila et al. (2001b) showed that this species can be an important component of epiphytic assemblages on macroalgae in the northwestern Mediterranean, and may represent up to 77 % of the total epiphytic abundance, corresponding to densities between 2 and 7573 cells g^{-1} fresh weight of macroalgae. Koike et al. (1998) have reported much lower *P. lima* densities of 2 to 4 cells g^{-1} fresh weight of macroalgae in the cold waters of the Sanriku coast in northern Japan, in an area where shellfish become toxic in the absence of *Dinophysis* spp. *P. lima* clones isolated from Sanriku were found to produce OA in the range of 0.3 to 1.3 pg $cell^{-1}$, and the authors suggest this organism may be involved in local shellfish toxicity. *P. lima* has also been suggested as the most likely source of DSP contamination of shellfish in Nova Scotia, Canada by Lawrence et al. (1998). Recently, Levasseur et al. (2003) have found that *P. lima* is a common epiphyte in two lagoons of the Magdalen Islands in the Gulf of St. Lawrence, Canada. These authors have reported average *P. lima* abundances of 4060 and 2969 cells g^{-1} dry epibiont weight from June to October in each lagoon and peak abundances of 9671 and 8087 cells g^{-1} dry epibiont weight. Although DSP toxins were not detected in mussel flesh obtained from the Magdalen lagoons, *P. lima* cells were found in the digestive glands of the mussels at abundances of up to 167 cells g^{-1} wet digestive gland weight (Levasseur et al. 2003). The authors suggest that the number of *P. lima* cells consumed by the mussels was insufficient to result in the accumulation of toxins in this shellfish.

Prorocentrum lima has been observed in UK waters (Lebour, 1925, Dodge, 1982), but has not to date been directly tested for toxicity. In 2000 during the current study, *P. lima* cells were found in association with macroalgae in the Fleet lagoon and single cells were isolated and a number of monoclonal cultures established in the laboratory. Following these preliminary observations of the widespread abundance of *P. lima* as an epiphyte on macroalgae in the Fleet lagoon, a more detailed survey of this species was undertaken from April to August in 2002 by Foden, as part of a MSc Project (Foden, 2002). Foden (2002) showed that seagrass (*Zostera* spp. and *Ruppia* spp.) and macroalgae species of Chlorophyta, Rhodophyta and Phaeophyta from the more central area of the lagoon (stations 2 to 6 of the present study) were colonized by densities of *P. lima* that varied from 1000 to 7542 cells g^{-1} fresh weight of macro-autotrophs during July and August 2002. An increasing density of *P. lima* was observed from April to August, and this was likely to be in response to the spring/summer growth of macroalgae and seagrass and the consequential increase in available substratum. Furthermore, Foden detected DSP

toxin concentrations in the range of 0.13-1.77 pg OA $cell^{-1}$ and 0.23-6.31 pg DTX-1 $cell^{-1}$ in concentrated natural populations of *P. lima* from the Fleet.

DSP toxins have been detected by mouse bioassay in the Pacific oyster (*Crassostrea gigas*) cultivated in the Fleet lagoon collected on 31/7/00 and 15/8/00 by CEFAS on behalf of the Foods Standards Agency and this caused the closure of the area for harvesting (Diaz-Fiunte, Food Standards Agency, pers. comm.). The DSP toxin complex is produced by species of both the planktonic dinoflagellate *Dinophysis* and epi-benthic dinoflagellate *Prorocentrum*. *Dinophysis* spp. cells have not been reported previously in the plankton of this lagoon, and were not observed in plankton samples collected during the two weekly/monthly survey of the Fleet undertaken as part of this research in 2000/2001 (see chapter 3). This strongly suggests that *P. lima* is the source of DSP toxins that have been occasionally detected in oysters from the Fleet lagoon.

In this chapter, morphological features (cell length and width and number of valve and marginal pores) observed under the scanning electron microscope and toxin profiles are reported on 20 strains of *P. lima* isolated from the Fleet lagoon. OA and DTX-1 were also quantified in oysters collected from the Fleet during 2001. Furthermore, results of three growth experiments are reported, when toxin concentrations, pigment composition, growth rate and nitrate and phosphate consumption from the growth media were determined for two strains during their growth in batch cultures.

6.2 – Results

6.2.1 OA and DTX-1 biotoxins in shellfish from the Fleet lagoon

The Pacific oyster *Crassostrea gigas* has been cultured in the Fleet since 1987. All the oysters analyzed during this study were acquired from the Abbotsbury oyster farm, located between our sampling stations 6 and 7 (see methods section 2.1.7.2). Oysters are cultivated inside gauze bags either lying on the bottom of the lagoon or over small metal racks, around 20 cm above the sediment (Fig. 6.1). These racks provide an artificial reef colonized by different macroalgae species, that grow around the area of the oyster farm and even on top of the bags containing oysters. The macroalgae provide substratum that have the potential to be colonized by epiphytic dinoflagellates like *P. lima*.

Detectable but low OA and DTX-1 concentrations were found in shellfish from the Fleet lagoon during 2001. OA concentrations ranged from 1.0 ng g^{-1} oyster flesh on 16/10/01 to 5.8 ng g^{-1} on 24/7/01, while lower DTX-1 concentrations, up to 1.4 ng g^{-1} were detected (Table 6.1). OA was the main biotoxin compound measured in the oyster

flesh. These low concentrations are well below the DSP regulatory level of 160 ng g^{-1} for whole shellfish tissue, generally used to safeguard shellfish consumers from symptoms of DSP. Although some differences were observed between the concentrations of OA and DTX-1 in oysters grown on the lagoon bed or on top of racks, no consistent pattern was found. Cockles (*Cerastoderma* sp.) collected from the sediment at station 4 showed the same range of OA and DTX-1 concentrations as measured in oyster flesh.

Figure 6.1: Abbotsbury oyster farm in the Fleet lagoon (low tide), showing the oyster bags on top of racks.

Table 6.1: OA and DTX-1 concentrations (ng g^{-1} shellfish flesh; wet weight) in shellfish samples collected from the Fleet lagoon from July to November 2001. On 14/8/01 no oyster samples from racks were collected.

Sample	Date	Origin	OA	DTX-1
Oysters	24/7/01	lagoon bed	4.1	1.4
		rack	5.8	0.0
	14/8/01	lagoon bed	3.6	0.1
		rack	-	-
	25/901	lagoon bed	5.5	0.0
		rack	4.8	0.0
	16/10/01	lagoon bed	7.1	0.2
		rack	1.0	0.0
	27/11/01	lagoon bed	1.5	0.0
		rack	2.9	0.4
Cockles	14/8/01	lagoon bed	4.9	1.2
	25/9/01	lagoon bed	1.6	0.1

Filters used in the filtration of water samples (1 – 2 L) collected from each of the sampling sites in the lagoon on each sampling date in 2000 and 2001 were stored frozen for later toxin extraction and analysis. All filters from station 5 (2000) were processed

initially as *P. lima* cells were slightly more abundant in the plankton samples from this site (Table 6.8). No OA and DTX-1 toxins were detected in seston samples collected from station 5 during 2000 however, which indicates that the source of DSP toxins detected in oysters and cockles from the Fleet lagoon is not a planktonic organism. Filters prepared from other sites were subsequently not processed for toxin analysis.

6.2.2 Morphology and toxicity of 20 *P. lima* strains isolated from the Fleet lagoon

Morphology of P. lima using scanning electron microscopy

A number of strains of *P. lima* were isolated from the Fleet lagoon, as described in the Methods chapter (section 2.2.1). Table 6.2 presents the adopted code of each one of the 21 strains that were successfully maintained in culture, the date and place of isolation from the Fleet lagoon and the type of substratum cells were isolated from.

Table 6.2: *P. lima* strains used in the present study, date of isolation, place of origin in the Fleet lagoon and sample type.

Strain	Date	Place of origin	Sample type
2.3b	May, 2000	Chickerell Point (st. 5)	eel grass
2.3d	May, 2000	Chickerell Point (st. 5)	eel grass
2.5a	May, 2000	Chickerell Point (st. 5)	eel grass
2.5d	May, 2000	Chickerell Point (st. 5)	eel grass
2.5e	May, 2000	Chickerell Point (st. 5)	eel grass
2.5f	May, 2000	Chickerell Point (st. 5)	eel grass
2.5g	May, 2000	Chickerell Point (st. 5)	eel grass
2.5h	May, 2000	Chickerell Point (st. 5)	eel grass
2.8c	June, 2000	Cloud's Hill (st. 2)	eel grass
2.8e	June, 2000	The Narrows (st. 6)	macroalgae
2.8g	June, 2000	The Narrows (st. 6)	macroalgae
2.9a	June, 2000	The Narrows (st. 6)	macroalgae
2.9b	June, 2000	The Narrows (st. 6)	macroalgae
2.9c	June, 2000	The Narrows (st. 6)	macroalgae
2.9d	June, 2000	The Narrows (st. 6)	macroalgae
2.10c	June, 2000	Langton Herring (st. 3)	net sample
3.4g	July, 2000	The Narrows (st. 6)	macroalgae
3.10d	September, 2000	Chickerell Point (st. 5)	macroalgae
3.10e	September, 2000	Chickerell Point (st. 5)	macroalgae
3.10f	September, 2000	Chickerell Point (st. 5)	macroalgae
3.10g	September, 2000	Chickerell Point (st. 5)	macroalgae

P. lima strains isolated from the Fleet lagoon were oblong to ovate in shape, broad in the middle region and narrow at the anterior end, with some cells showing relatively straight sides (Fig. 6.2). Both valves have a smooth surface, are concave in the centre and the right valve has the flagellar pore plates fitted into a V-shaped anterior area. The left valve margin exhibits a flat ridge. Marginal pores are uniform in size and evenly spaced around the periphery of the valve. Valve pores are arranged unevenly and the centre of the

valve is devoid of pores (Faust, 1991). All strains studied in this work presented these typical morphological characteristics.

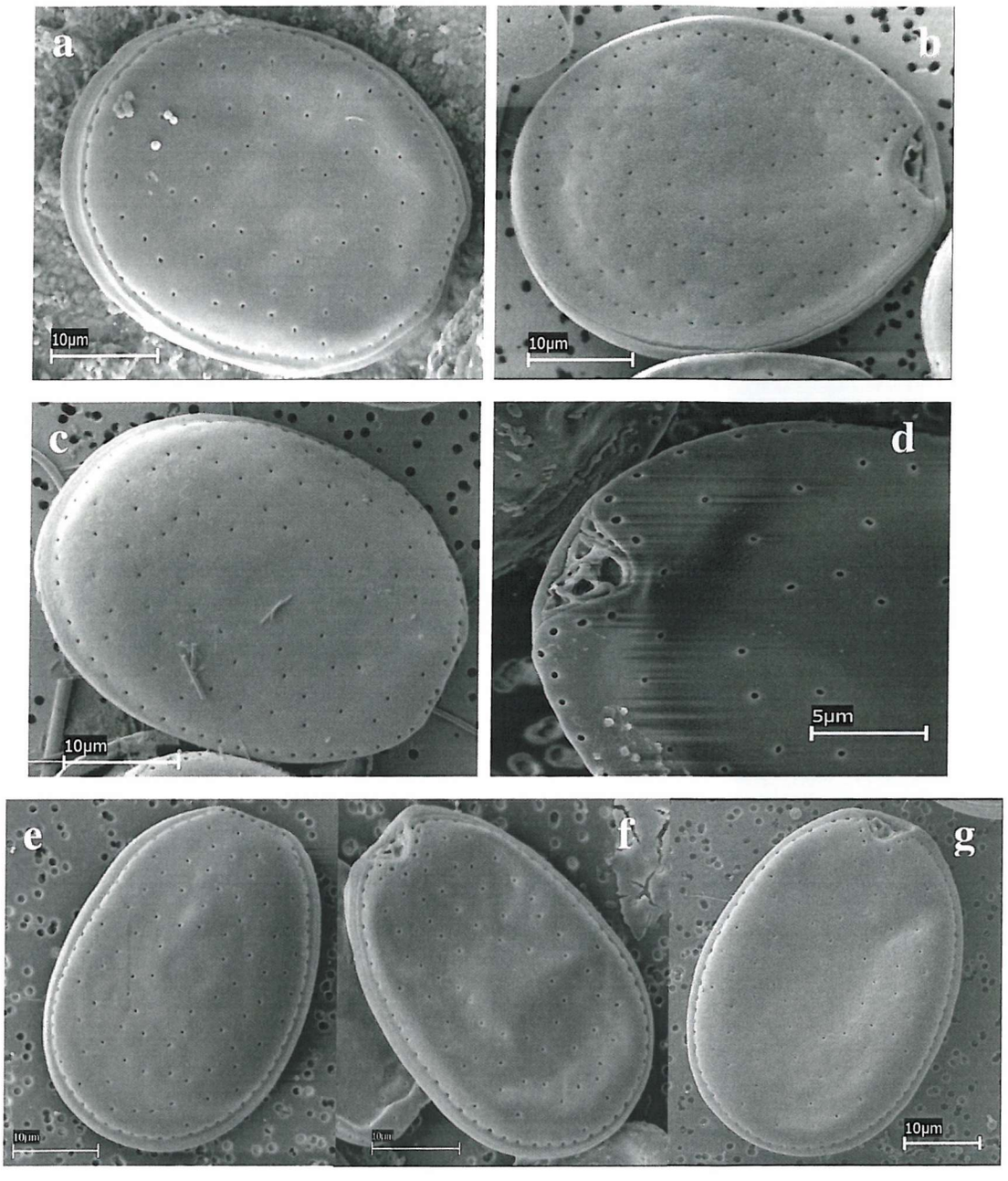


Figure 6.2: SEM micrographs of *P. lima* strains. a) 2.3b, b) 2.5a, c) 2.5h, d) 2.5d (showing details of the flagellar pore plates), e) 3.10g (left valve), f) 3.10g (right valve), g) 2.5h.

Cell length varied from 29.4 to 45.6 µm and cell width varied from 23.1 to 32.1 µm (Table 6.3). Cell length and width are within the range previously described for *P. lima*, except for the length of strain 3.4g, that is slightly smaller than the value of 31 µm described by Faust (1991) as the lower limit for the clones she examined. Variation in cell shape expressed as the length-to-width ratio ranged between 1.2 and 1.5 (Table 6.3). The number of valve and marginal pores was determined in only 8 strains and ranged between

48 and 69 (valve pores) and 47 and 60 (marginal pores). These numbers agree well with the ones reported by Faust (1991) for *P. lima*.

Table 6.3: Comparison of cell size and number of valve and marginal pores of *P. lima* strains isolated from the Fleet lagoon (average ± standard deviation), nd: not determined.

Strain	Size of cells			Number of pores	
	Length (μm)	Width (μm)	Ratio (L/W)	Valve	Marginal
2.3b	35.3 ± 2.9	27.2 ± 2.4	1.3	58 ± 7	47 ± 6
2.3d	42.2 ± 2.3	28.2 ± 1.4	1.5	nd	nd
2.5a	38.7 ± 1.7	29.5 ± 2.4	1.3	65 ± 5	50 ± 4
2.5d	41.3 ± 1.3	29.7 ± 1.1	1.4	48 ± 7	59 ± 6
2.5e	39.0 ± 4.3	29.2 ± 2.8	1.3	nd	nd
2.5f	39.0 ± 1.6	29.6 ± 0.6	1.3	nd	nd
2.5g	nd	nd	nd	nd	nd
2.5h	45.6 ± 1.5	32.1 ± 0.9	1.4	65 ± 7	70 ±16
2.8c	35.9 ± 1.3	27.5 ± 1.1	1.3	nd	nd
2.8e	40.0 ± 2.8	29.4 ± 1.8	1.4	nd	nd
2.8g	43.5 ± 0.6	31.5 ± 0.9	1.4	64 ± 6	60 ± 3
2.9a	32.7 ± 1.8	26.0 ± 2.3	1.3	nd	nd
2.9b	38.7 ± 2.4	27.4 ± 1.8	1.4	nd	nd
2.9c	38.6 ± 1.8	28.2 ± 1.8	1.4	nd	nd
2.9d	39.3 ± 1.4	29.8 ± 1.2	1.3	58 ± 7	57 ± 3
2.10c	34.4 ± 3.0	27.5 ± 1.5	1.2	nd	nd
3.4g	29.4 ± 3.3	24.7 ± 2.2	1.2	nd	nd
3.10d	36.7 ± 1.8	29.3 ± 1.5	1.3	63 ± 12	50 ± 3
3.10e	42.1 ± 3.0	27.8 ± 1.7	1.5	nd	nd
3.10f	32.2 ± 1.6	23.1 ± 1.7	1.4	nd	nd
3.10g	43.4 ± 2.0	30.5 ± 1.2	1.4	69 ± 5	60 ± 3

P. lima OA and DTX-1 content

OA and DTX-1 were the principal biotoxins measured in all 20 strains studied. DTX-2 was not detected in any strain (Table 6.4). The toxins produced by *P. lima* cells were consistent with the toxin profile found in shellfish collected from the Fleet. Fig. 6.3 shows selected chromatograms of strain 3.10e.

OA concentrations varied between 0.4 to 17.1 pg OA cell^{-1} whilst DTX-1 varied from 0.4 to 11.3 pg DTX-1 cell^{-1}. The ratio OA/DTX-1 ranged from 0.14 to 3.20, reflecting the diverse toxin profile among different strains. Strains 2.5e, 2.8c, 2.9a, 3.4g, 3.10d, 3.10f and 3.10g produced more OA than DTX-1 while strains 2.5a, 2.5d, 2.5f, 2.5g, 2.5h, 2.8g, 2.9b and 2.9d produced more DTX-1 than OA. Other strains produced similar amounts of each compound (Table 6.4). For the few strains that were analyzed in replicas (2.3b, 2.10c and 3.4g), quite large differences between OA and DTX-1 concentrations per cell were detected. This may be partially due to differences in the cellular contents of these compounds between cells and to difficulties in quantifying *P. lima* cells, as they often form clumps of cells. However, the replicas of cultures 2.10c and 3.4g originated from the same culture flask, with the volume divided between two centrifuge tubes. The difference

observed between OA and DTX-1 content per cell between replicas of these two cultures can be related to the fact that the culture media poured into the second centrifuge tube (second replica) was used to dislodge *P. lima* cells that were attached to the bottom of the culture flask. This was done through pipetting media against the bottom of the flask to create a flow to detach these cells. It is possible that the mixing created by this procedure caused the release of OA and DTX-1 by the *P. lima* cells that were left in the culture flask. This would explain the differences in toxin content per cell between replicas and the increased amounts of extracellular toxins measured in the growth media of these two cultures (see Table 6.6).

Table 6.4: Toxin composition (OA and DTX-1) of several *P. lima* strains isolated from the Fleet lagoon, including cell numbers in each sample and the day the culture was harvested.

Strain	pg OA cell^{-1}	pg DTX-1 cell^{-1}	ratio OA/DTX-1	cell numbers (cells mL^{-1})	day harvested
2.3b	3.13	2.69	1.16	29922	21
2.3b	0.57	0.53	1.08	20156	23
2.3d	1.56	1.35	1.16	5625	23
2.5a	0.67	1.72	0.39	12969	29
2.5d	1.22	6.05	0.2	31563	23
2.5e	0.59	0.41	1.43	37917	23
2.5f	0.69	3.18	0.22	48359	23
2.5g	0.42	2.21	0.19	16563	32
2.5h	1.54	4.14	0.37	6250	23
2.8c	4.48	1.40	3.20	65781	23
2.8e	7.72	10.88	0.71	12813	32
2.8g	1.42	2.62	0.54	11563	23
2.9a	5.64	3.4	1.66	64844	23
2.9b	0.94	6.82	0.14	7656	32
2.9c	1.67	1.38	1.21	13047	32
2.9d	1.15	4.25	0.27	13333	23
2.10c	1.4	1.1	1.27	43125	26
2.10c	2.02	3.03	0.67	72500	26
3.4g	17.13	11.29	1.52	39219	26
3.4g	5.32	2.54	2.1	46406	26
3.10d	9.41	6.57	1.43	107813	55
3.10f	4.00	2.66	1.5	3750	32
3.10g	4.07	2.61	1.56	14219	23

Chromatograms of some of the *P. lima* strains were screened to look for the presence of a selection of DTX-4 diol esters, OA diol ester and DTX-1 diol ester using LC/MS selected ion monitoring (see Methods section 2.2.3.2) (Table 6.5). DTX-4, DTX-4+O and DTX-4+CH_2+2O were detected in all the strains screened while other ester derivatives were found in only some of them. OA and DTX-1 diol esters were not found in any of the strains chosen.

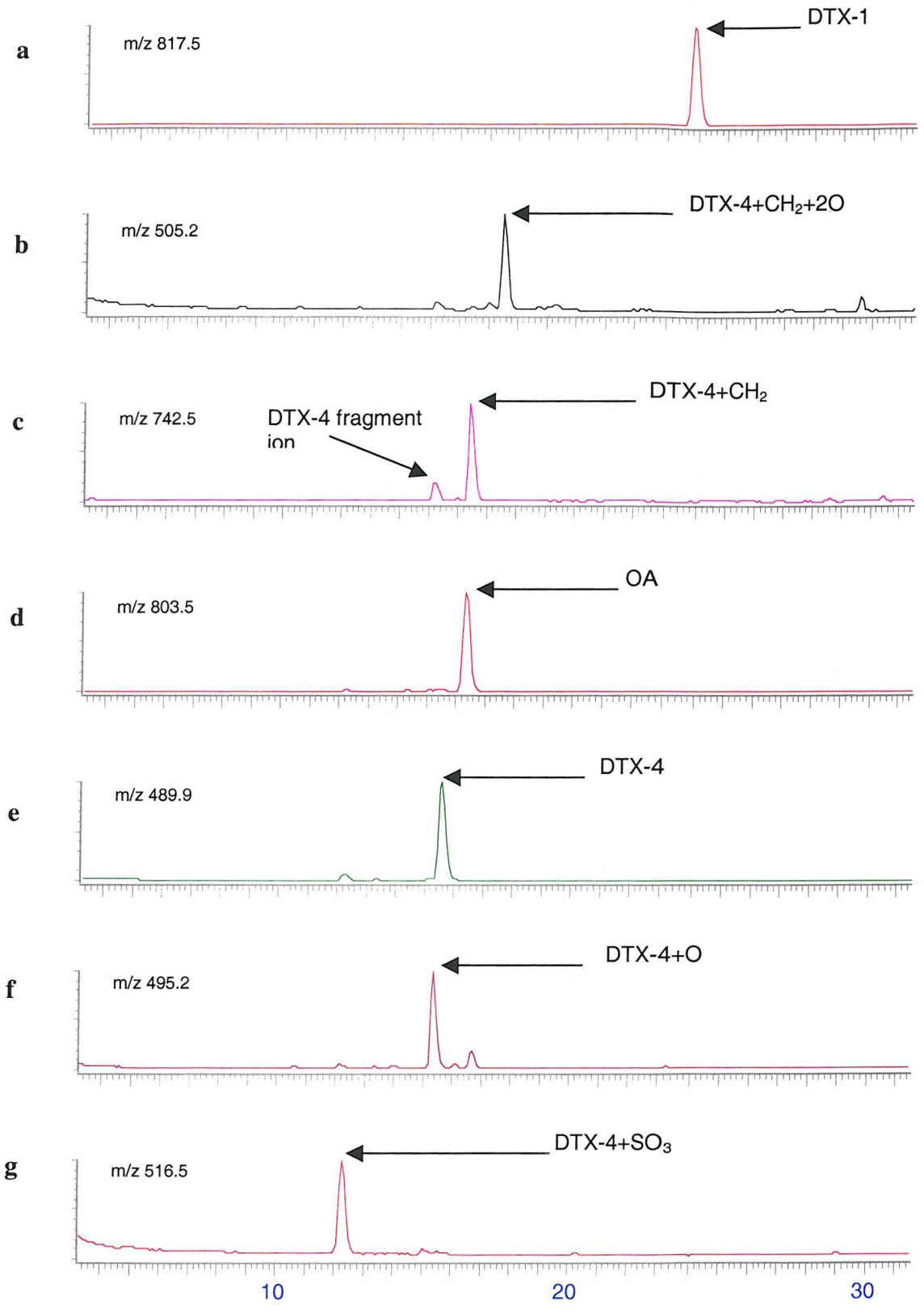


Figure 6.3: Selected ion chromatograms showing ions of a) DTX-1 $[M-H]^-$, b) DTX-4+CH_2+2O $[M-3H]^{3-}$, c) DTX-4+CH_2 $[M-2H]^{2-}$, d) OA $[M-H]^-$, e) DTX-4 $[M-3H]^{3-}$, (ion m/z 735.3 $[M-2H]^{2-}$ was also used as a confirmatory ion, data not presented), f) DTX-4+O $[M-3H]^{3-}$ and g) DTX-4+SO_3 $[M-3H]^{3-}$.

Table 6.5: A selection of DTX-4, OA and DTX-1 diol esters in several *P. lima* strains isolated from the Fleet lagoon. PRES indicates presence and ND is not detected.

Diol ester (in chromatographic elution order)	*P. lima* strain										
	m/z*	2.3b	3.10d	2.5d	2.9a	2.9d	2.5a	3.10g	3.4g	2.8e	3.10f
DTX-4+SO_3+O	521.9	PRES	ND	ND	ND	ND	ND	ND	ND	ND	ND
DTX-4+SO_3+2O	527.7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
DTX-4+SO_3	516.5	PRES	ND	PRES	ND	ND	PRES	ND	ND	ND	ND
DTX-4+O	495.2	PRES	PRES	PRES	PRES	PRES	PRES	PRES	PRES	PRES	PRES
DTX-4	489.9+735.3	PRES	PRES	PRES	PRES	PRES	PRES	PRES	PRES	PRES	PRES
OA	803.5	PRES	PRES	PRES	PRES	PRES	PRES	PRES	PRES	PRES	PRES
DTX-4+CH_2	742.3	PRES	PRES	PRES	ND	PRES	PRES	ND	ND	ND	PRES
DTX-4+CH_2+2O	505.2	PRES	PRES	PRES	PRES	PRES	PRES	PRES	PRES	PRES	PRES
DTX-4+CH_2+SO_3+2O	798.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
DTX-4+2O	500.5	ND	PRES	ND	ND	PRES	PRES	PRES	PRES	PRES	PRES
DTX-1	817.5	PRES	PRES	PRES	PRES	PRES	PRES	PRES	PRES	PRES	PRES
OA diol ester	308.5+463.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
DTX-1 diol ester	313.2+470.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

* m/z, mass to charge ratio value of ion(s) used to monitor for presence or absence of each diol ester.

OA and DTX-1 toxins in the culture media

OA and DTX-1 were also detected in the culture media. Quite variable concentrations were found, ranging from zero to 59581 pg OA mL^{-1} and zero to 94626 pg DTX-1 mL^{-1}. Culture media from cultures of the same strain, e.g. 2.10c and 3.4g were pooled and analyzed together, although each culture yielded two cell pellets that were analyzed as replicas. The percentage of toxins present in the culture media was very low for nearly all strains, except for strains 2.3b, 2.9c, and 2.10c (Table 6.6), as discussed previously. In the culture media used to grow some other strains, e.g. 2.9a, 3.4g and 3.10d, high amounts of OA and DTX-1 were detected, although the percent of toxins in the growth media was low relative to the amount inside the cells. DTX-4 was not observed in the culture media. Increased OA and DTX-1 concentrations in the growth media of strains 3.4g e 2.10c are likely to be related to the procedure used to detach cells from the bottom of the culture flask, as discussed above.

Table 6.6: Toxin concentrations (OA and DTX-1) in the culture media used to grow several *P. lima* strains isolated from the Fleet lagoon and percent of extracellular OA or DTX-1 relative to extra+intracellular concentrations.

	Culture media		% in Culture media			
	pg OA mL^{-1}	pg DTX-1 mL^{-1}	pg OA mL^{-1}	pg DTX-1 mL^{-1}	cell numbers (cells mL^{-1})	day harvested
2.3b	37147.8	4254.7	28.4	5.0	29922	21
2.3b	0	0	0	0	20156	23
2.3d	0	0	0	0	5625	23
2.5a	0	0	0	0	12969	29
2.5d	8.9	23.9	0	0	31563	23
2.5e	129.6	136.9	0.6	0.9	37917	23
2.5f	0	0	0	0	48359	23
2.5g	1.1	12.4	0	0	16563	32
2.5h	0	0	0	0	6250	23
2.8c	2.1	0	0	0	65781	23
2.8e	117.9	244.6	0.1	0.2	12813	32
2.8g	3.9	0	0	0	11563	23
2.9a	2389.6	2071.1	0.6	0.9	64844	23
2.9b	0.9	3.7	0	0	7656	32
2.9c	14521.7	13380.6	40	42.7	13047	32
2.9d	0	0	0	0	13333	23
2.10c	59581	94625.8	49.8	66.6	43125	26
2.10c	59581	94625.8	28.9	30.1	72500	26
3.4g	9390.1	6002.7	1.4	1.3	39219	26
3.4g	9390.1	6002.7	3.7	4.9	46406	26
3.10d	4270.9	2487.2	0.4	0.4	107813	55
3.10f	229.0	155.5	2.4	1.5	3750	32
3.10g	3.6	1.6	0	0	14219	23

Culture 3.10d was harvested after 55 days of growth and was probably during stationary growth phase, as indicated by the high cell number (Table 6.6). It is possible that the higher amounts of both OA and DTX-1 detected in the culture media of this culture are related to cell debris that were not retained by centrifugation, as increased rates of cell death are expected to occur at this stage of a batch culture. The increased extracellular content of OA and DTX-1 may also be explained by a leakage of these compounds from senescent cells. However, it is unclear why culture 2.9c released high amounts of toxins to the growth media.

6.2.3 - Growth experiments

Growth rate

All growth experiments were performed at the same temperature (17 °C) and 90 µmoles photon flux m^{-2} s^{-1}, however during experiments 1 and 2 the light period was of 12 h (photoperiod 12 hours light, 12 hours dark) and in experiment 3 it was increased to 16 hours. Strains 2.5a and 2.9a were chosen for the growth experiments as they had less tendency to form clumps of cells, making it easier to produce more reproducible cell counts.

Experiment 1 was performed using strain 2.5a grown in two, 1 L flasks that were sub-sampled every 3 days during the first 16 days, and then every 6 days from day 16 to day 28. These cultures showed very low growth rates, of 0.022 day^{-1} on average (based on cell numbers from day 1 to 28) and an exponential growth phase was not clearly distinguished (Fig. 6.4a). The average number of cells on day 1 was 900 cells mL^{-1} while on day 28 the average density of the two cultures was 1556 cells mL^{-1}. The cell counts showed quite large standard errors on some occasions, partly due to the patchy distribution of *P. lima*, as it tends to form aggregates, and also possibly due to the poor condition of cells disturbed by frequent turbulent agitation during experiment 1.

During the second experiment, *P. lima* strain 2.5a was grown using several small flasks to reduce the disturbance caused by the stirring of the culture before each sub-sampling. A total of 12 flasks were arranged in the incubator at the same distance from the light source to ensure a uniform light environment. Two flasks were sacrificed every 6 days of sampling and the others were not disturbed. These cultures showed an initial lag phase of 11 days and the growth rate during the exponential phase (from day 12 to 30) was of .023 day^{-1} (Fig. 6.4b). The final cell yield of the culture, on day 30 although higher than in experiment 1 was still low (2596 cells mL^{-1}).

During experiment 3, a different strain (2.9a) was cultured in 14 flasks over 45 days and the photoperiod was increased to 16 hours light and 8 hours dark. The mean initial cell density was 2444 cells mL^{-1} and the cultures final cell yield reached a mean value of 48308 cells mL^{-1} (Fig. 6.4c). The exponential growth phase lasted from day 1 to day 25, no lag phase was observed and the mean growth rate was 0.11 day^{-1}. The characteristic sigmoid growth pattern typically exhibited by phytoplankton grown in batch culture was observed.

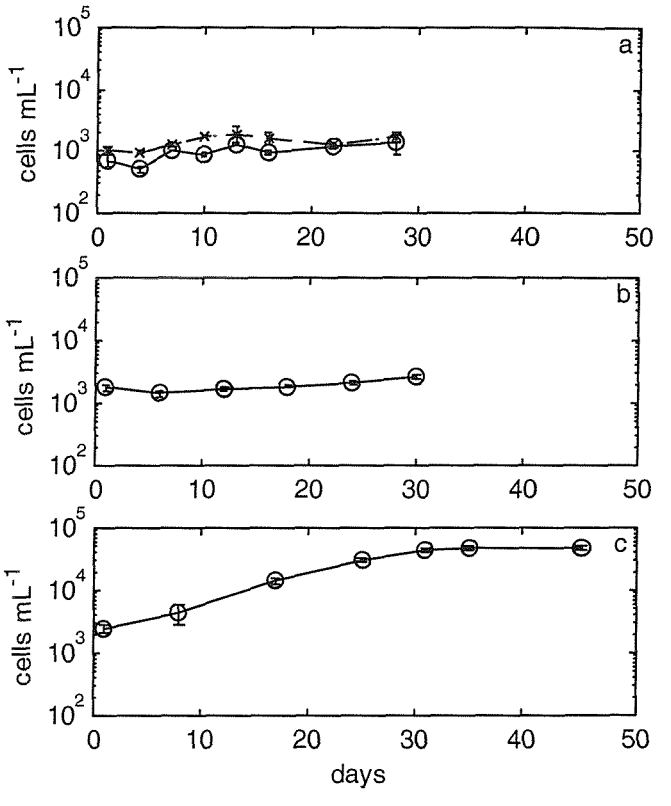


Figure 6.4: *P. lima* growth curves during experiment 1, strain 2.5a (a), experiment 2, strain 2.5a (b) and experiment 3, strain 2.9a (c). In (a) continuous line with open circles corresponds to culture 1 and dashed line with x to culture 2, each point is a mean of two measurements and error bars corresponds to ± 1 standard difference. In (b) and (c) each point is a mean of four measurements, two for each culture flask. Error bars corresponds to ± 1 standard deviation.

Nitrate and phosphate consumption from the growth media

Nitrate and phosphate concentrations in the L2 - media are respectively 883 μM and 36.3 μM. The values measured on the first day of each experiment were within ± 5 % error for nitrate and within ± 10 % error for phosphate (838 μM and 33 μM in experiment 1, 913 μM and 37.7 μM in experiment 2 and 842 μM and 38 μM in experiment 3), showing a good agreement between the "theoretical" concentrations and the values measured.

During experiment 1, nitrate and phosphate concentrations in the growth media decreased towards the end of the experiment, and on day 28 concentrations were 700 μM of nitrate (Fig. 6.5a) and 26 μM of phosphate (Fig 6.6a). However, on some days an apparent increase in concentration was observed. These increases in the concentrations of nitrate and phosphate are most likely caused by cellular lyses and/or the release of phosphate and nitrite to the media, as cells showed very poor growth, probably due to the frequent turbulent mixing of the cultures before each sampling. The N:P ratio of the L-2 media is 24:1, however the nitrate to phosphate draw-down ratio during experiment 1, calculated from the slope of the linear regression of nitrate and phosphate concentrations in the growth media from day 1 to 28, was 19 ($p < 0.01$) (Fig. 6.7a).

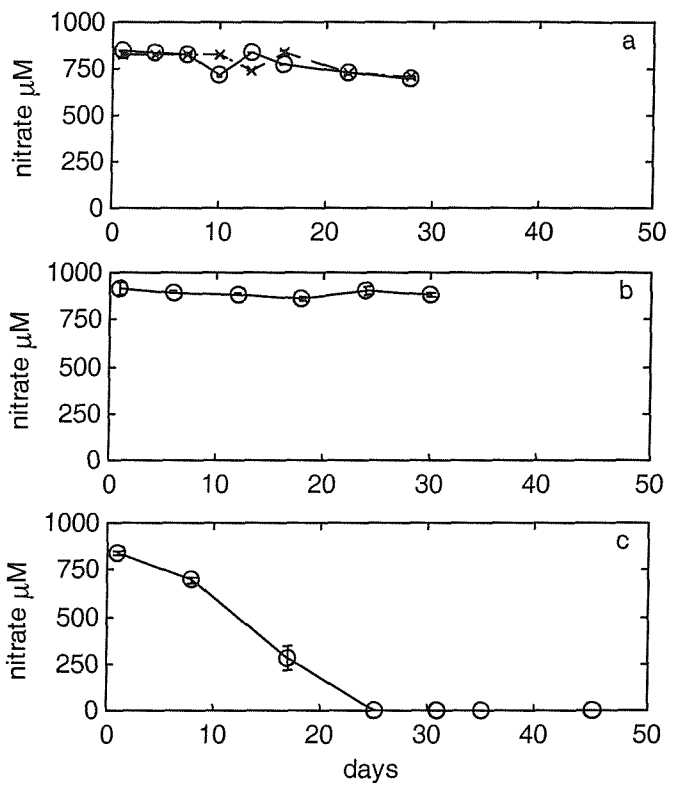


Figure 6.5: Changes in nitrate concentrations in the growth media. In (a) continuous line with open circles corresponds to culture 1 and dashed line with x to culture 2, each point is a mean of two measurements and error bars corresponds to ± 1 standard difference. In (b) and (c) each point is a mean of four measurements, two for each culture flask. Error bars corresponds to ± 1 standard deviation.

During experiment 2 and 3, nutrient concentrations were measured in 12 different flasks, instead of being followed in the same culture flask. In this case, apparent increases

in the concentrations of nitrate and phosphate during the growth experiment may have been caused by differential consumption in the different culture flasks.

In experiment 2, nitrate concentrations changed only slightly, and on day 30 concentrations were 885 μM of nitrate (Fig. 6.5b) and 29 μM of phosphate (Fig 6.6b). Between days 1 and 6 and 12 and 18, the consumption of nitrate was of 3.6 μM day^{-1}, although a high standard error was observed for the period between days 1 and 6 due to a very high nitrate value (940 μM) measured on day 1 in one of the culture flasks. Maximum phosphate consumption of 1.1 μM day^{-1} was observed from day 1 to 6, and lower of 0.2 μM day^{-1} followed from day 6 to 18.

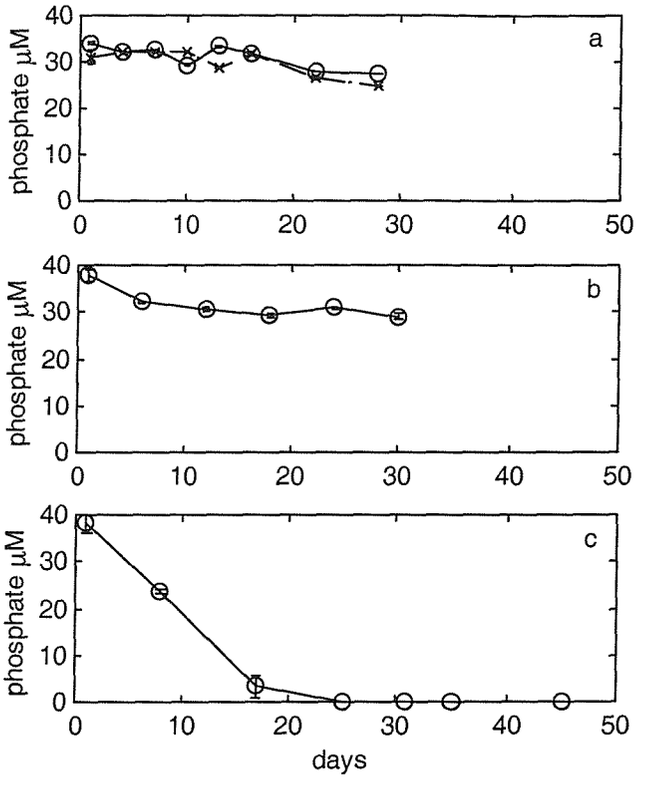


Figure 6.6: Changes in phosphate concentrations in the growth media. In (a) continuous line with open circles corresponds to culture 1 and dashed line with x to culture 2, each point is a mean of two measurements and error bars corresponds to ± 1 standard difference. In (b) and (c) each point is a mean of four measurements, two for each culture flask. Error bars corresponds to ± 1 standard deviation.

The nitrate to phosphate (N:P) draw-down ratio during experiment 2, calculated from the slope of the linear regression of nitrate and phosphate concentrations in the growth media from day 1 to 18, was 4.0 ($p < 0.01$) (Fig. 6.7b). Considering the N:P draw-down ratio calculated from the difference in nitrate and phosphate concentrations between each sampling date (Table 6.7), it can be seen that it was closer to the Redfield N:P ratio

(16:1) from day 12 to 18. Before and after this period cells were probably accumulating phosphate.

Table 6.7: Mean consumption of nitrate and phosphate (μM day^{-1}) by *P. lima* strain 2.5a during experiment 2 and strain 2.9a during experiment 3. Numbers in brackets represent standard differences of two means. Where numbers are not shown, it is because an increase in nutrient concentrations was observed.

	Time range (days)	Nitrate	Phosphate	N:P ratio
2nd Exp.	1-6	3.6 (8.3)	1.1 (0.0)	3.3
	6-12	2.0 (0.6)	0.2 (0.1)	10.0
	12-18	3.6 (1.7)	0.2 (0.2)	18.0
	18-24	-	-	-
	24-30	2.7 (1.8)	0.3 (0.1)	9.0
3rd Exp.	1- 8	20.9 (1.1)	2.1 (0.3)	9.9
	8-17	46.4 (9.5)	2.3 (0.3)	20.2
	17-25	34.7 (10.2)	0.4 (0.4)	86.8
	25-31	-	0.0 (0.0)	-
	31-35	0.2 (0.0)	0.0 (0.0)	-
	35-45	0.0 (0.0)	0.0 (0.0)	-

During experiment 3, both nitrate and phosphate were exhausted from the growth media by day 25, coinciding with the end of the exponential growth phase. After this day concentrations of both nutrients reached values close to 1 μM (Fig. 6.5c and 6.6c). Maximum consumption of nutrients was 46.4 μM nitrate day^{-1} and 2.3 μM phosphate day^{-1} during mid exponential growth phase (from day 8 to day 17) of *P. lima* (Table 6.7). The mean N:P draw-down ratio, calculated from the slope of the linear regression of nitrate and phosphate concentrations in the growth media from day 1 to 17, was 16.5 ($p < 0.01$) (Fig. 6.7c). *P. lima* was accumulating phosphate during the initial 8 days of growth, as shown by the N:P draw-down ratio calculated for this period (Table 6.7). From day 8 to 17 the N:P draw-down ratio was closer to Redfield, while from day 17 to 25 cells were consuming more nitrogen relative to phosphate, as shown by the very high N:P draw down ratio (Table 6.7).

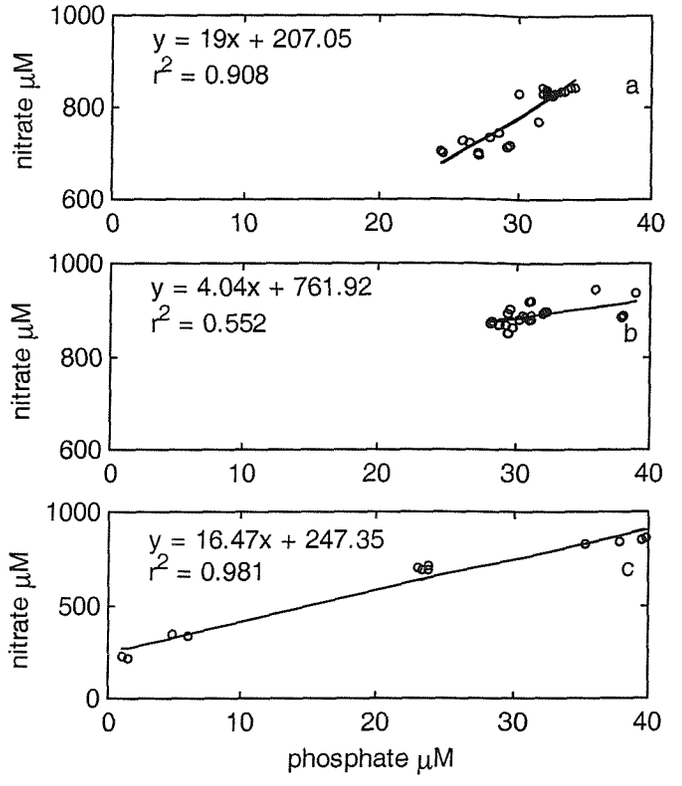


Figure 6.7: Nitrate against phosphate concentrations in the growth media during experiment 1, n = 32, $p < 0.01$ (a), experiment 2, n = 24, $p < 0.01$ (b) and experiment 3, n = 12, $p < 0.01$ (c), showing the equation of the line and correlation coefficients. Two replicas for each of two culture flasks on every sampling date were included (four points per date). In experiment 3 only the three first sampling dates were included because nitrate and phosphate concentrations had decreased to less than 1 µM after this day. Note change of y scale in figure c.

Pigments

Chlorophyll-a concentrations: Comparison between HPLC and fluorometric determinations

Chlorophyll-a (chl-a) was measured by fluorometry and by HPLC. Comparisons between HPLC chl-a concentrations and fluorometric chl-a concentrations are shown in Fig. 6.8. The HPLC method is considered to be the most accurate method for measuring pigment composition (Mantoura et al. 1997), so comparisons between HPLC and fluorometric chl-a determinations will be made using the HPLC values as standard. In experiment 1, chl-a data showed large error bars and the noise in this data hampered any attempt to find a relationship between the two datasets (Fig. 6.8a), as shown by the low correlation coefficient ($r^2 = 0.38$, $p < 0.05$) and slope (0.62) of the regression line.

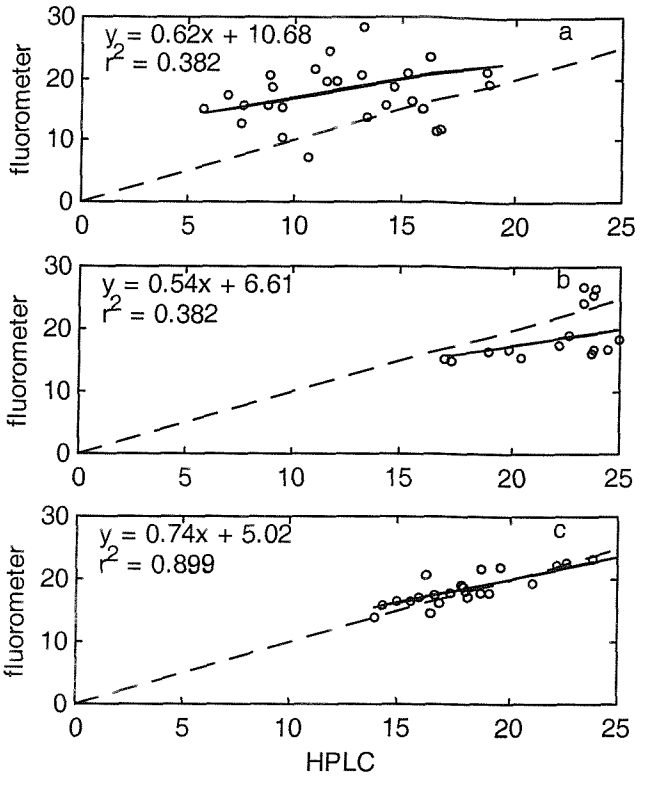


Figure 6.8: Chl-a values (pg cell^{-1}) measured by fluorescence against chl-a values measured by HPLC during experiment 1, n = 28, $p < 0.05$ (a), experiment 2, n = 24, p not significant at the 5% level (b) and experiment 3, n = 29, $p < 0.01$ (c). The solid lines represent the trendline for each set of data and the equation for this line and correlation coefficient are shown. The dashed line represents the 1:1 agreement line. Two replicas for each of two culture flasks of every sampling date were included.

In experiment 2, a more consistent trend was observed, but the correlation coefficient of the regression line ($r^2 = 0.38$) and slope (0.54) were still low (Fig. 6.8b) and the correlation coefficient was not significant at the 5% level. For most of the measurements, the fluorometer underestimated chl-a concentrations. During experiment 3, a better agreement was observed, as shown by the regression correlation coefficient ($r^2 = 0.90$, $p < 0.01$) and slope (0.74), with data points scattered around the 1:1 line (Fig. 6.8c). Mantoura et al. (1997) reported a correlation coefficient of 0.85 when comparing chl-a concentrations determined fluorometrically according to the method of Holm-Hansen et al. (1965) and by HPLC. However, the fluorometric method used in this work (Welschmeyer, 1994) optimizes a new combination of lamps and optical filters to improve the selective detection of chlorophyll-a in the presence of chlorophyll-b and phaeopigments. Chlorophyll-b was not a problem in this study, though, as dinoflagellates do not produce it. In this case, the poor agreement between the two methods in experiment 1 may have been influenced by the poor physiological state of the cells.

Pigment composition

Chl-a, peridinin and diadinoxanthin were the major pigments produced by *P. lima* strains 2.5a and 2.9a during the three experiments. Minor pigments observed were chl-c_1+c_2, chl-c_3, diatoxanthin and β-carotene. A peak at the retention time of 19'butanoyloxyfucoxanthin and with similar absorption spectra was also observed and identified as this pigment.

During experiment 1, chl-a concentrations per cell showed an initial decreasing trend until day 7, followed by an increase, peaking on day 16 (Fig. 6.9a and 6.10a). This same pattern was also observed for all other pigments except chl-c_3 (Fig. 6.10), that had large error bars (Fig. 6.10e). In general, cells in culture 2 showed higher pigment concentrations per cell, although values were quite similar on day 1. 19'buta was not detected in cells cultured during experiment 1.

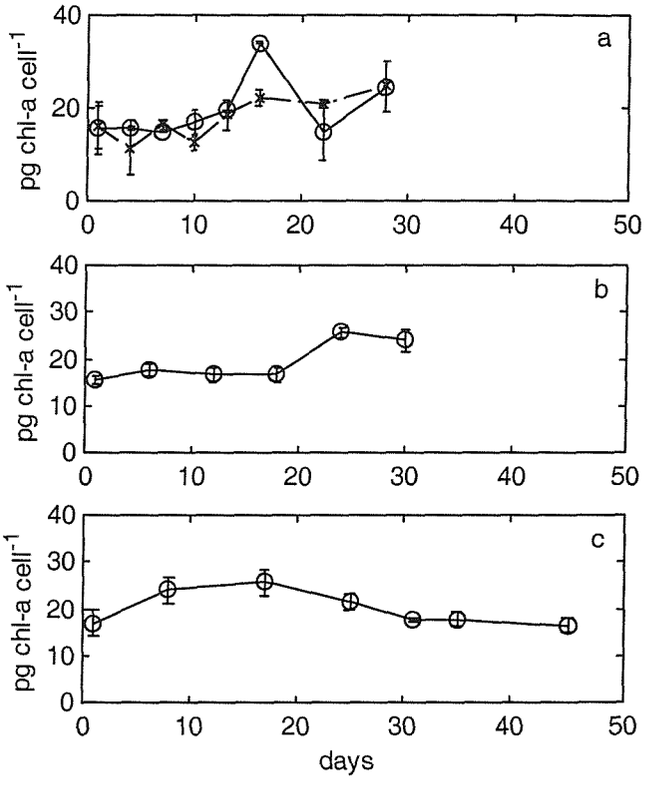


Figure 6.9: *P. lima* chl-a concentrations (measured by fluorometry) during experiment 1, strain 2.5a (a), experiment 2, strain 2.5a (b) and experiment 3, strain 2.9a (c). In (a) continuous line with open circles corresponds to culture 1 and dashed line with x to culture 2, each point is a mean of two measurements and error bars corresponds to ± 1 standard difference. In (b) and (c) each point is a mean of four measurements, two for each culture flask. Error bars corresponds to ± 1 standard deviation.

During experiment 2, all pigments showed the same tendency of an initial increase from day 1 to 6, and then remained stable, finally peaking on day 30 (Fig. 6.9b and 6.11a-

h). Chl-c_1+c_2 showed a marked increase on day 30 (Fig. 6.11f). In experiment 3 all pigments but diatoxanthin peaked during the exponential growth phase and then decreased from day 17 (Fig. 6.9c and 6.12). Maximum concentrations of chl-c_1+c_2, chl-c_3 and 19'butanoyloxyfucoxanthin did not exceed 0.8 pg cell^{-1}. Diatoxanthin peaked on day 45, and an inverse pattern was observed between concentrations of diadinoxanthin and diatoxanthin (Fig. 6.12c,d).

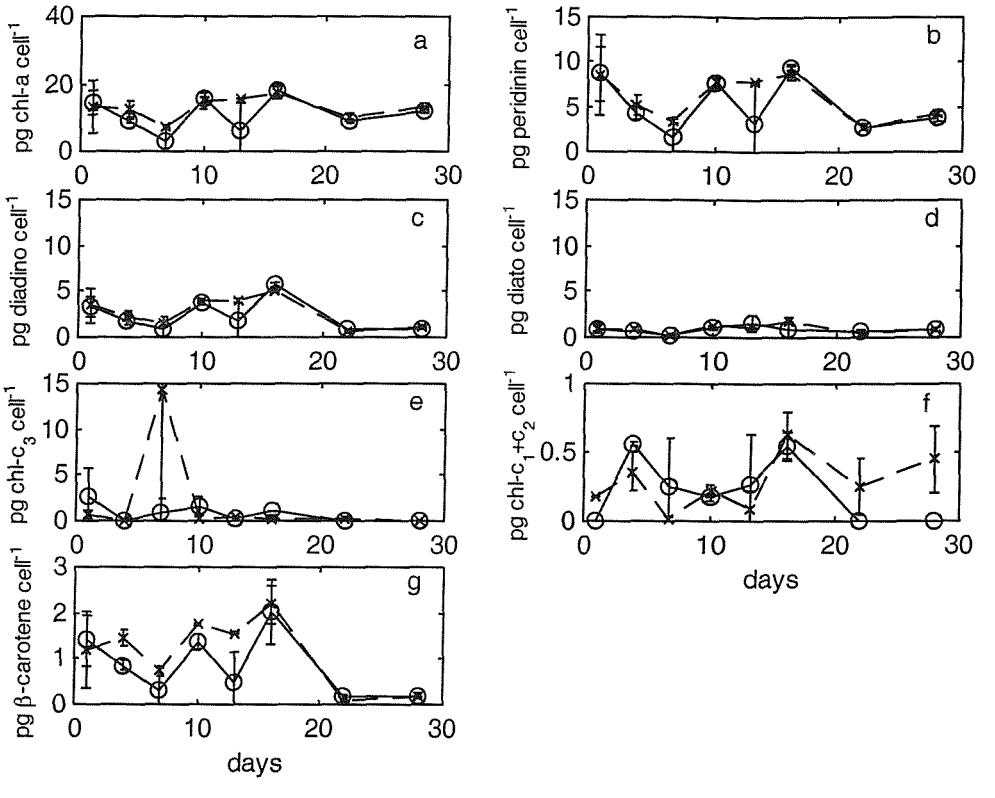


Figure 6.10: Changes in pigment contents of *P. lima* (measured by HPLC) during experiment 1, strain 2.5a (a) chlorophyll-a, (b) peridinin, (c) diadinoxanthin, (d) diatoxanthin, (e) chlorophyll-c_3, (f) chlorophyll-c_1+c_2, and (g) β-carotene. Continuous line with open circles corresponds to culture 1 and dashed line with x to culture 2, each point is a mean of two measurements. Error bars corresponds to ± 1 standard difference.

The ratio of chl-a to peridinin was around 2 in all three experiments, except towards the end of experiment 1, when it increased to 3.4 (Fig. 6.13). The chl-a to diadinoxanthin ratio was quite variable during experiment 1, ranging between 1.1 and 16.2 (Fig. 6.14a) and the replicates showed distinctly different values on day 22. Diadinoxanthin concentrations showed a marked decrease from day 16 to day 22 (from 5.5 to 0.79 pg cell^{-1}), that was more pronounced than the chl-a decrease. These changes in concentrations were reflected in an increase in the chl-a to diadinoxanthin ratio. During experiment 2, this ratio was 6.4 on day 1, then decreased gradually to around 4 from day 24 to 30 (Fig. 6.14b). In experiment 3, the chl-a to diadinoxanthin ratio was quite constant

around 4 from day 1 to 35, increasing to 5.6 on day 45, due to a pronounced decrease in diadinoxanthin concentrations on this day (Fig. 6.14c).

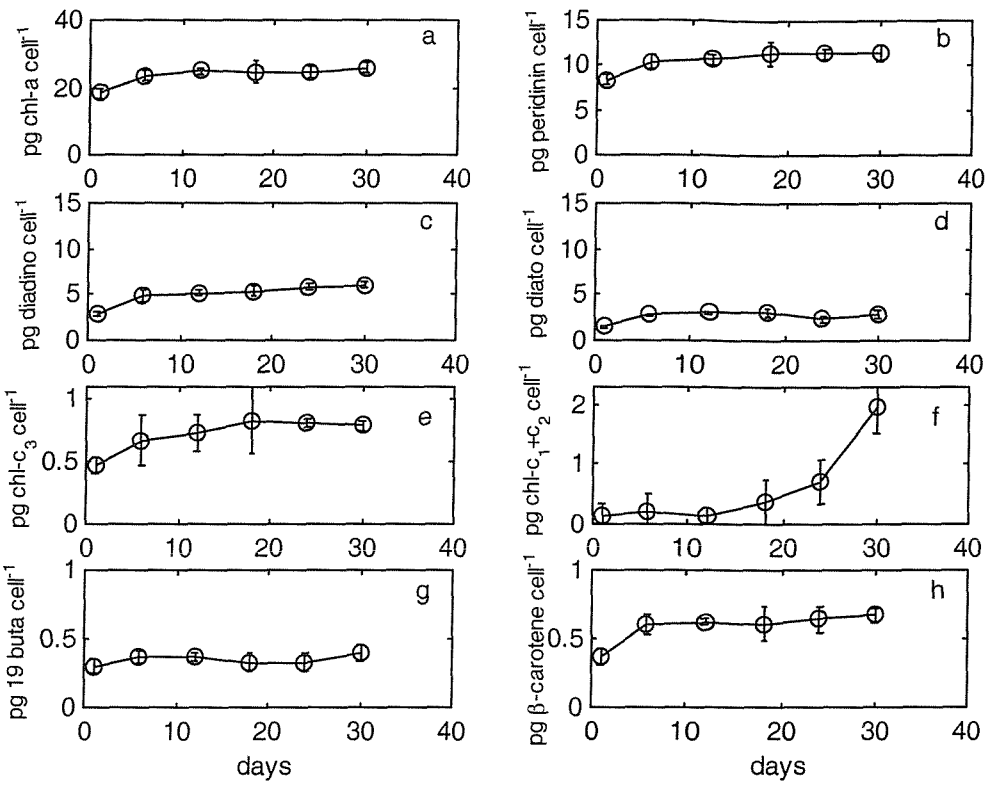


Figure 6.11: Changes in pigment contents of *P. lima* (measured by HPLC) during experiment 2, strain 2.5a (a) chlorophyll-a, (b) peridinin, (c) diadinoxanthin, (d) diatoxanthin, (e) chlorophyll-c_3, (f) chlorophyll-c_1+c_2, (g) 19'buta, and (h) β-carotene. Each point is a mean of four measurements, two from each culture flask. Error bars corresponds to ± 1 standard deviation.

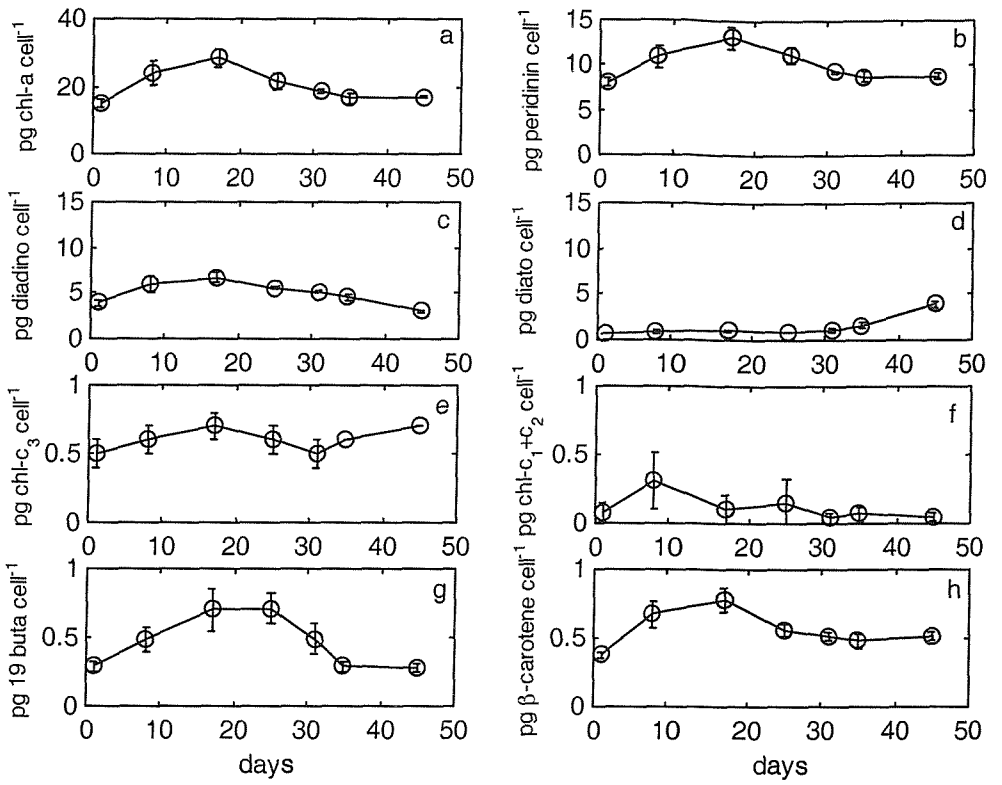


Figure 6.12: Changes in pigment contents of *P. lima* (measured by HPLC) during experiment 3, strain 2.9a (a) chlorophyll-a, (b) peridinin, (c) diadinoxanthin, (d) diatoxanthin, (e) chlorophyll-c_3, (f) chlorophyll-c_1+c_2, (g) 19'buta, and (h) β-carotene. Each point is a mean of four measurements, two for each culture flask. Error bars corresponds to ± 1 standard deviation.

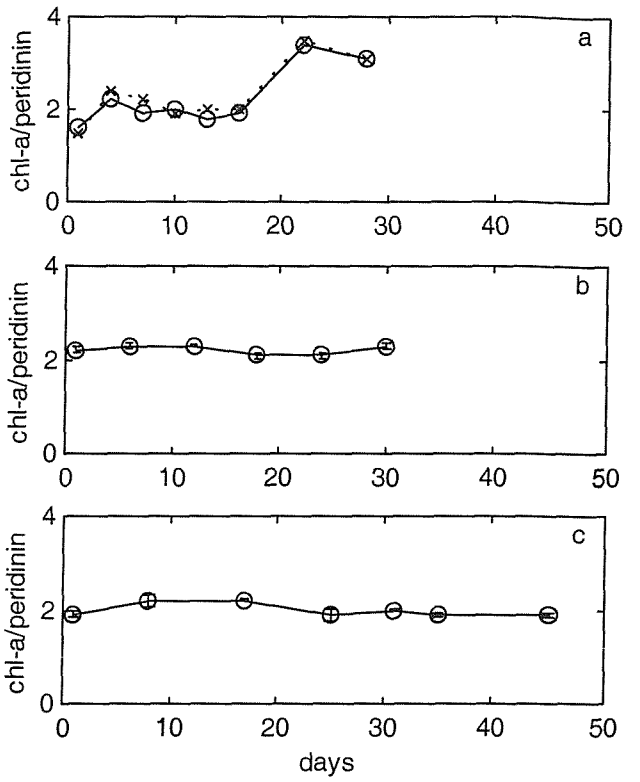


Figure 6.13: Chl-a to peridinin ratio during experiment 1, strain 2.5a (a), experiment 2, strain 2.5a (b) and experiment 3, strain 2.9a (c). In (a) continuous line with open circles corresponds to culture 1 and dashed line with x to culture 2, each point is a mean of two measurements and error bars corresponds to ± 1 standard difference. In (b) and (c) each point is a mean of four measurements, two for each culture flask. Error bars corresponds to ± 1 standard deviation.

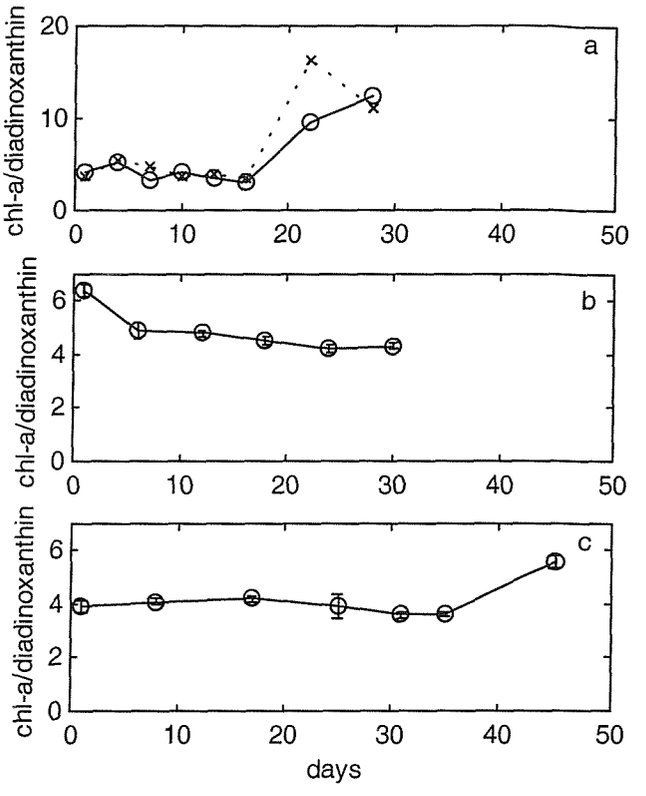


Figure 6.14: Chl-a to diadinoxanthin ratio during experiment 1, strain 2.5a (a), experiment 2, strain 2.5a (b) and experiment 3, strain 2.9a (c). In (a) continuous line with open circles corresponds to culture 1 and dashed line with x to culture 2, each point is a mean of two measurements and error bars corresponds to ± 1 standard difference. In (b) and (c) each point is a mean of four measurements, two for each culture flask. Error bars corresponds to ± 1 standard deviation. Note change on y scale on figure a.

OA and DTX-1 toxins along the growth curve

OA and DTX-1 concentrations were quantified during the three growth experiments. During experiment 1, both OA and DTX-1 showed the same temporal trend, peaking on day 4, then decreasing towards day 28 (Fig. 6.15a, b). OA varied between 0.2 and 0.9 pg cell^{-1} and DTX-1 between 0.5 and 2.6 pg cell^{-1}. The OA/DTX-1 ratio remained quite constant around 0.3-0.4 throughout experiment 1. OA and DTX-1 previously detected in this strain were 0.7 pg OA cell^{-1} and 1.7 pg DTX-1 cell^{-1} and the OA/DTX-1 ratio was 0.4 (Table 6.4), showing good agreement between these values.

During experiment 2, OA and DTX-1 content of strain 2.5a varied between 0.3 and 0.8 pg OA cell^{-1} and 1.7 and 3.7 pg DTX-1 cell^{-1}. Concentrations per cell showed a slight increase on days 24 and 30, although the toxin content of one of the cultures was lower on day 30 (Fig. 6.15c,d). The OA/DTX-1 ratio remained quite constant around 0.2-0.3 throughout experiment 2. Samples from one of the cultures from day 24 showed wide

error bars for both OA and DTX-1, that might have been caused by differential cell distribution in each of these sub-samples, probably due to cell clumping.

In experiment 3, higher OA and DTX-1 concentrations were produced by strain 2.9a, consistent with results shown in table 6.4 (5.6 pg OA $cell^{-1}$ and 3.4 pg DTX-1 $cell^{-1}$). Toxin content varied between 2.0 and 12.2 pg OA $cell^{-1}$ and 1.8 and 12.5 pg DTX-1 $cell^{-1}$ (Fig. 6.15). Toxin concentrations per cell decreased initially from day 1 to 8, then remained quite constant during the exponential growth phase, increasing during late exponential – stationary phase, from day 25 to 45, peaking on the last day, although one of the cultures harvested on day 45 showed lower toxin content then the other (Fig. 6.15e,f). The OA/DTX-1 ratio remained constant at 1.25-1.31 until day 17, and then progressively decreased from late exponential growth phase towards day 45 when it was 0.94.

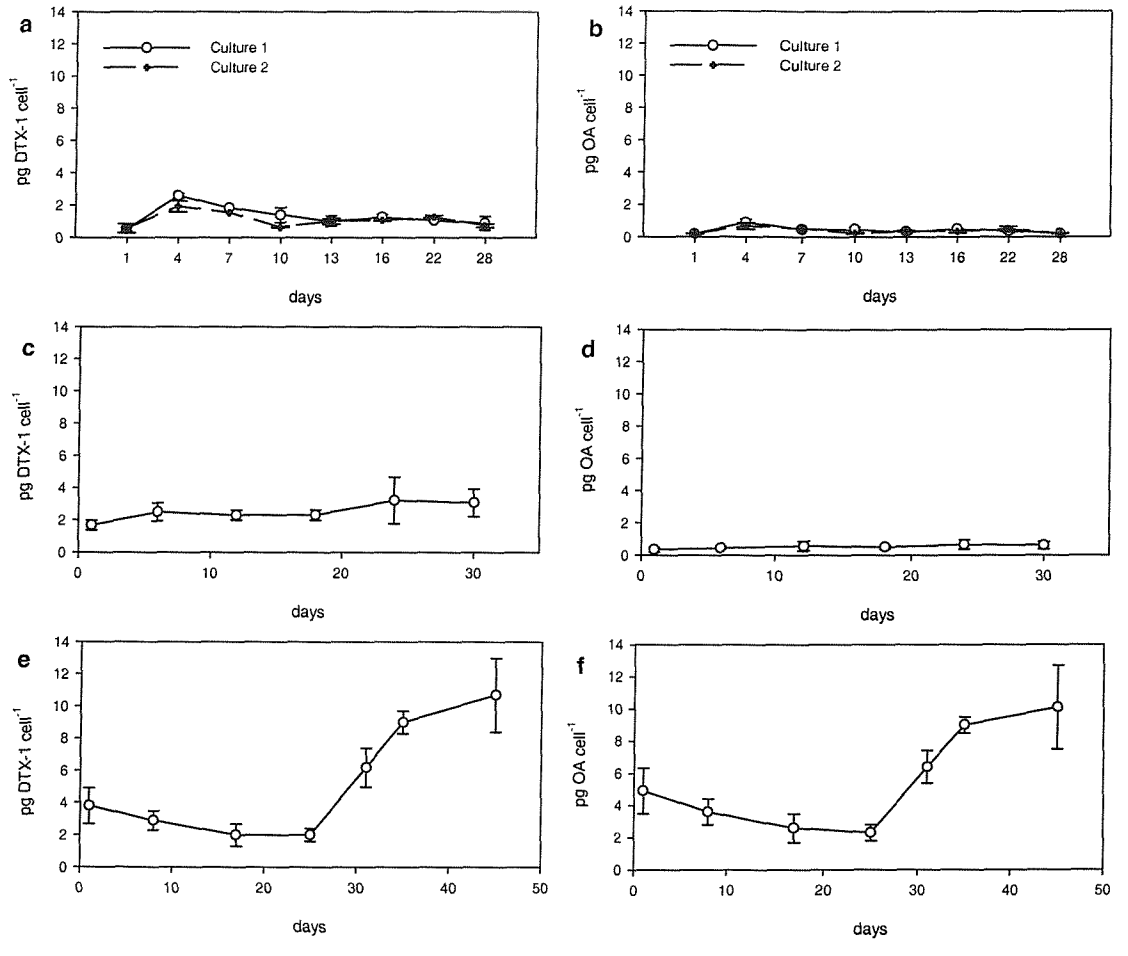


Figure 6.15: *P. lima* DTX-1 and OA content during experiments 1, strain 2.5a (a,b), experiment 2, strain 2.5a (c,d) and experiment 3, strain 2.9a (e,f). In (a) each point is a mean of two measurements and error bars corresponds to ± 1 standard difference. In (b) and (c) each point is a mean of four measurements, two for each culture flask. Error bars correspond to ± 1 standard deviation. Note change on x scales.

6.3 Discussion

OA and DTX-1 biotoxins in shellfish from the Fleet

OA and DTX-1 concentrations measured in oysters and cockles from the Fleet lagoon during 2001 were far below the widely accepted DSP regulatory level of 160 ng g^{-1} for shellfish whole tissue. This contrasts with DSP concentrations in oysters from the Fleet collected on 31/7/00 and 15/8/00, when samples tested positive on the DSP mouse bioassay (mouse bioassay as Yasumoto et al. 1978). The amount of toxin required to kill two of three mice (20 g) within 24 hours has been defined as one mouse unit (MU), and corresponds to 4 μg OA, 3.2 μg DTX-1 and 5 μg of DTX-3 (James et al. 2001). Taking this into account, shellfish collected from the Fleet on 31/7 and 15/8/2000 probably contained DSP toxins in concentrations greater than that corresponding to 1 MU. It is unfortunate that shellfish samples were not routinely collected from the oyster farm during 2000, and it was not possible to quantify and identify the DSP toxins present during the toxic event of summer 2000 in the Fleet.

During 2000 and 2001 *Dinophysis* spp. cells were not observed in the phytoplankton community of the Fleet lagoon, although lugol preserved plankton samples and concentrated net samples were collected fortnightly during 2000 and monthly during 2001. *P. lima* is an epiphytic-benthic dinoflagellate that is occasionally found in the plankton. Table 6.8 shows *P. lima* abundances in water samples collected during the surveys of the Fleet in 2000 and 2001. *P. lima* cells were mostly observed in association with macroalgae samples collected from the lagoon during these years. Subsequently a study by Foden (2002) showed that *P. lima* colonizes macroalgae around the area of the oyster farm in the Fleet, with cell abundances between 107 (in May) and 852 (in July) cells g^{-1} fresh weight macroalgae. In the mid Fleet, even higher densities of *P. lima* were observed, that varied from 1000 to 7542 cells g^{-1} fresh weight macro-autotrophs during July and August 2002 (Foden 2002). Furthermore, Foden was able to measure OA and DTX-1 concentrations in the range 0.1-1.8 pg OA $cell^{-1}$ and 0.2-6.3 pg DTX-1 $cell^{-1}$ for concentrated natural epiphytic populations of *P. lima* from the Fleet, although these toxins were not detected in oysters collected from the oyster farm in summer 2002.

Bauder et al. (2001) have demonstrated that *P. lima* can act as a vector for DSP toxins in shellfish, using laboratory experiments when *P. lima* was offered as the only food for bay scallops (*Argopecten irradians*). In their experiments, DSP toxin concentrations in bay scallop tissues surpassed accepted closure levels within 18 hours of exposure to *P. lima*.

Table 6.8: *P. lima* abundances in water samples collected from the Fleet lagoon in 2000 and 2001.

***P. lima* abundances (cells mL^{-1}) during 2000**							
Date	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7
18-May			1		9		
1-Jun	43	0	0				
14-Jun	0	0	0	0	4	0	0
27-Jun	0	0	0	0	2	2	0
11-Jul	0	0	2	0	4	0	0
25-Jul	0	0	0	0	4	0	0
8-Aug	0	0	0	0	1	0	0
22-Aug	0	0	0	0	0	0	0
5-Sep	0	0	0	0	3	0	0
19-Sep	0	8	0	0	1	0	0
3-Oct	4	0	0	0	0	0	0
17-Oct	0	0	0	0	0	0	0
4-Nov		0	0	0	0	0	0

***P. lima* abundances (cells mL^{-1}) during 2001**							
Date	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	
6-Apr	0	0				0	
23-May	0	0	0			0	
14-Jun	0	0	0	0	1.1	0	
24-Jul	0	0	0	0	0	0	
14-Aug	0	0	2.1	0	0	0.6	
25-Sep	0	0	0	0	0.2	0	
16-Oct	0	0	0.8	0.2	0.4	0	
27-Nov	0	0	0	0.2	0.2	0	

In the Fleet lagoon, many factors may account for the low concentration of toxins in shellfish even in the presence of abundant toxic *P. lima* cells associated with macroalgae. As *P. lima* is an epiphyte, it may become more readily available for filter feeding shellfish only when it is physically dislodged from macroalgae, by wind or tidal mixing events and thus enters the water column. Moreover, interannual variability is likely to occur within the epiphytic assemblage, and some species may be more abundant in one year than in another, as was observed for planktonic dinoflagellates (see chapter 3).

Meteorological conditions during sampling days of 25/7, 8/8 and 22/8/2000 and 24/7 and 14/8/2001 were of fine weather and calm conditions. Comparing meteorological data from the Portland meteorological station for the months that Foden (2002) showed a maximum abundance of *P. lima* (July and August) shows that July 2001 was wetter than the same month in 2000, while similar rainfall was observed in August 2000 and 2001 (see Fig. 3.27). Pronounced rainfall in July 2001 may account for more *P. lima* cells being dislodged from macroalgae, but may also contribute to the washing of cells out of the lagoon and to potentially lower growth rates as less light was available. From early to mid

July 2000 strong winds (NW to SE) were observed on the direction along the Fleet, aligned with the mouth of the lagoon, peaking at 11 m s^{-1}. In July 2001 strong winds were not as frequent, and the peak wind of 10 m s^{-1} (NW to SE) had a shorter duration time (see Fig. 3.28 and 3.29). Wind conditions were quite similar in August for both years (see Fig. 3.28 and 3.29). More constant strong winds may have contributed to the detachment of *P. lima* cells from seagrass and seaweeds, making them more available for shellfish. This might have caused the shellfishery closure during summer 2000 in the Fleet.

In the Gulf of St Lawrence, Levasseur et al. (2003) reported that a decrease of attached *P. lima* cells appeared to be related to vertical mixing caused by strong winds of 10 m s^{-1} that favored the development of waves within the lagoons. Also, strong winds physically removed the macroalgae that served as substrate for *P. lima*. Interestingly, Levasseur et al. (2003) observed that *P. lima* cells were frequently found in mussel digestive glands, in spite of the low *P. lima* abundances in the water column of the lagoons in the Gulf of St Lawrence, suggesting that they were available for ingestion. On the Sanriku coast of Japan, shellfish become contaminated with DSP toxins in the absence of *Dinophysis* spp. *P. lima* from the area was shown to produce OA and is now though to be involved in shellfish toxicity (Koike et al. 1998). In Nova Scotia, Canada, *P. lima* has been put forward as the most likely source of DSP toxicity (Lawrence et al. 1998).

Most DSP outbreaks have been related to the presence of *Dinophysis* spp. in the water column, as in the rias of Vigo, Spain (Bravo et al. 2001), Portugal (Vale and Sampayo, 1999), Japan (Suzuki et al. 1999) and New Zealand (Rhodes and Syhre, 1995). However, not all DSP incidents clearly correlate to *Dinophysis* spp. presence, and OA or DTX-1 producing *P. lima* strains have now been isolated from diverse locations around the world. In the Fleet lagoon, no *Dinophysis* species were detected in preserved plankton samples collected during 2000 and 2001. The proximity that shellfish is grown to macroalgae in this shallow lagoon indicates that *P. lima* is the most likely source of DSP toxicity, as this study has established that strains locally isolated are able to produce both OA and DTX-1. There are no previous reports of toxic *P. lima* strains isolated from coastal UK waters. However, it is possible that this epiphytic dinoflagellate may cause shellfish toxicity elsewhere around the coast, particularly in shallow areas where shellfish are cultivated close to seaweeds or *Zostera* spp. beds; or in areas where shellfish is grown in rafts that provide a habitat for the growth of macroalgae colonized by *P. lima*. It would be desirable to include the quantification of attached *P. lima* cells in a monitoring program aiming to detect early warning signs of potential DSP toxicity. This would be particularly

interesting in areas where DSP events occur without correlation to *Dinophysis* spp. presence in the water column and where shellfish is cultivated close to macroalgae.

OA and DTX-1 content of P. lima strains from the Fleet lagoon

Several isolates of *P. lima* from different geographical areas have been shown to be toxic, from coastal waters of Canada (Jackson et al. 1993), Australia (Morton and Tindall, 1995), Spain (Bravo et al. 2001), New Zealand (Rhodes and Syhre, 1995) and Japan (Murakami et al. 1982, Koike et al. 1998). Differences in the DSP toxin profile were found between strains however. A Japanese isolate of *P. lima* is capable of producing only OA, while OA and DTX-1 were identified in the Australian, Canadian and Spanish strains. The Spanish ones also produce low amounts of DTX-2. *P. lima* from New Caledonia (strain P6) was found to produce OA and 7-deoxy-OA, but not DTX-1 and isomers of OA (Holmes et al. 2001). *P. lima* strains from the Fleet lagoon were shown to produce both OA and DTX-1, but not DTX-2.

Regarding total toxicity, the strains from the Fleet produce OA concentrations ranging from 0.4 pg cell^{-1} to 17.1 pg cell^{-1} and DTX-1 ranging from 0.4 pg cell^{-1} to 11.3 pg cell^{-1}. Total toxicity, considering OA and DTX-1, varied between 1.0 and 28.4 pg cell^{-1}.

A number of naturally occurring derivatives of the parental toxin compounds have been observed in other *P. lima* strains. A *P. lima* strain from Canada stored most of the DSP toxins in the DTX-4 form, relative to OA and DTX-1 (Quilliam et al. 1996) and it has been proposed that the DTX-4-type sulfates are a means of safely harboring the lethal OA form in the cell (Hu et al. 1995a). However, Bravo et al. (2001) have shown that this does not hold for *P. lima* strains from northwestern Spain.

The toxin analyses from this work were performed on boiled *P. lima* cell pellets to preserve the native toxin profile of cells and in doing so, a number of diol esters derivatives of OA and DTX-4 could be identified in *P. lima* strains from the Fleet lagoon. However, when calculating the total toxicity of these strains, it should be noted that the boiling procedure does not account for the contribution of OA and DTX-1 derivatives that will be transformed to their parental toxins in the shellfish tissues. Therefore, the total toxicity of each isolate is likely to be higher than the one calculated based solely on OA and DTX-1 original concentrations.

Murakami et al. (1982) were the first to describe OA and DTX-1 production by a *P. lima* strain isolated from Tahiti Island, reporting 4 pg OA cell^{-1} (Table 6.9). More recently, Holmes et al (2001) have studied the DSP toxin profile of a New Caledonian *P. lima* strain and have reported OA and 7-deoxy-OA at concentrations of 1.5 and 15 pg cell^{-}

^{1} respectively after 18 days of growth, but no DTX-1 or isomers of OA were detected. These authors used two methods of analysis, one that favours enzymatic hydrolysis (freeze-thaw treatment of cell pellets) and one that denaturates enzymes involved in this process (boiling method) and were able to measure significantly higher OA concentrations (440 ± 57 %) when using the first method, indicating the conversion of OA derivatives to their parental toxins after cell disruption.

Table 6.9: OA and DTX-1 content of *P. lima* isolates from several geographic origins, including information about the toxin extraction procedure and detection method.

Origin	OA and DTX-1 content (pg cell^{-1})	Method employed	Reference
Tahiti islands	4.0 pg OA cell^{-1} no DTX-1	extraction at room temperature and detection by LC-MS	Murakami et al. (1982)
Heron island, Australia (17 clones)	1.3 - 5.9 pg OA cell^{-1} 4.0 – 12.0 pg DTX-1 cell^{-1}	extraction of freeze-dried cells and detection by HPLC-FD	Morton and Tindall (1995)
Mahone bay, Canada	8.0 pg OA+DTX-1 cell^{-1} at 5°C and 1.5 pg OA+DTX-1 cell^{-1} at 25°C	extraction at room temperature and detection by HPLC-FD and MS	Jackson et al. (1993)
Rangaunu Harbour, New Zealand	6.3 pg OA cell^{-1}	extraction at room temperature and detection by HPLC-FD	Rhodes and Syhre (1995)
Dry Tortugas, Florida	Maximum of 14.2 pg OA cell^{-1}, only trace concentrations of DTX-1	extraction at room temperature and detection by HPLC-FD	Tomas and Bader, (1993)
Sanriku coast, Japan (*P. lima* natural population)	0.3 – 1.3 pg OA cell^{-1} no DTX-1	extraction at room temperature and detection by HPLC-FD	Koike et al. (1998)
NW Spain (19 clones)	0.19 – 12.9 pg OA cell^{-1} 0 – 12.4 pg DTX-1 cell^{-1} 0 – 1.1 pg DTX-2 cell^{-1}	extraction of boiled and freeze/thaw cells and detection by HPLC-FD	Bravo et al. (2001)
Strain P6, New Caledonia	1.5 pg OA cell^{-1} no DTX-1 15.0 pg 7-deoxy-OA cell^{-1}	extraction of boiled and freeze/thaw cells and detection by LC/MS	Holmes et al. (2001)
Strain PRL-1, Gulf of California	19.0 pg OA+DTX-1 cell^{-1} OA:DTX-1 ratio of 1:2	mouse bioassay	Heredia-Tapia et al. (2002)
Fleet lagoon, UK (*P. lima* natural population)	0.1 – 1.8 pg OA cell^{-1} 0.2 – 6.3 pg DTX-1 cell^{-1}	extraction at room temperature and detection by LC-MS	Foden (2002)
Fleet lagoon, UK (20 clones)	0.4 – 11.3 pg OA cell^{-1} 0.4 – 17.1 pg DTX-1 cell^{-1}	extraction of boiled cell pellets and detection by LC-MS	this study

Bravo et al. (2001) reported total toxicity values for *P. lima* strains from northwestern Spain that varied between 2 and 28.6 pg cell^{-1}, considering the contribution of OA and DTX-2 esters to total toxicity.

Considering data presented in table 6.9, it is noteworthy that *P. lima* OA content per cell is remarkably similar among strains from different geographic origins. OA content per *P. lima* cell varies between 0.1 and 14.2 pg cell^{-1}, based on a comparison that includes strains from Europe, North America, Japan, Australia, New Zealand, Tahiti and New Caledonia (Table 6.9). OA was detected in all strains studied, however, DTX-1 was not

found in strains from Tahiti, Japan and New Caledonia (Table 6.9). DTX-1 was detected in concentrations that ranged from 0.2 to 17.1 pg cell^{-1} in *P. lima* strains from Europe, North America and Australia (Table 6.9). The concentration of OA and DTX-1 produced by *P. lima* strains from the Fleet lagoon is in the range of concentrations observed in other *P. lima* strains from elsewhere in the world. Moreover, the amount of OA and DTX-1 per cell reported for the *P. lima* natural populations from the Fleet lagoon by Foden (2002) is also similar to those detected in the isolated clonal cultures in this study.

Few of the studies reported in table 6.9 have applied the boiling procedure of *P. lima* cells before toxin extraction. Therefore, some biotransformations of sulfated derivatives and diol esters into OA may have happened after the cells were disrupted during toxin extraction. These comparisons indicate that some of the strains isolated from the Fleet are apparently more toxic than the ones studied by other authors.

Bouaicha et al. (2001) described the toxicity of *P. lima* from lagoons from four islands in the SW Indian Ocean through protein phosphatase inhibition assay, reporting a range in OA equivalent amount varying from 6261.3 ± 156.5 to 128.3 ± 17.2 ng mg^{-1} crude extract.

Other epi-benthic species of *Prorocentrum* are also capable of producing OA and related derivatives, although fewer strains of other *Prorocentrum* species have been studied. A strain of *Prorocentrum hoffmannianum* isolated from the U.S. Virgin Islands was shown to produce OA in the range of 3.5 – 20.0 pg OA cell^{-1} (Aikman et al. 1993) and 7.9 pg OA cell^{-1} were measured in *Prorocentrum concavum* from the British Virgin Islands (Dickey et al. 1990). In *Prorocentrum faustiae* from Heron Island, Australia, Morton (1998) reported the production of 4.2 pg OA cell^{-1} and 12.4 pg DTX-1 cell^{-1}, while *P. belizeanum* from Belize is able to produce 12.4 pg OA cell^{-1} (Morton et al. 1998). *P. arenarium* from the SW Indian Ocean and *P. maculosum* from the British Virgin Islands were also shown to produce OA (Ten-Hage et al. 2000, Zhou and Fritz, 1994). Interestingly, all epi-benthic *Prorocentrum* species shown to be toxic, produce OA and related compounds in concentrations of the same order of magnitude. In contrast, planktonic species of *Prorocentrum* such as *P. micans* and *P. minimum* do not produce these compounds.

Most of the OA and DTX-1 produced by *P. lima* strains isolated from the Fleet were measured from whole cell extracts and for most of the cultures no DTX-1 or OA was found in the culture media. The percentage of toxins present in the culture media was high only in cultures of strains 2.9c, 3.4g and 2.10c. The culture media of cultures 3.4g and 2.10c was used to detach *P. lima* cells attached to the bottom of the culture flasks using a

pipette. It is possible that the *P. lima* cells have released large amounts of toxins to the growth media when agitated during this procedure, which would also explain the differences between OA and DTX-1 content per cell between replicas of these cultures. Pan et al. (1999) have observed two periods of higher OA and DTX-1 concentrations in the culture media; the first associated with increased sampling turbulence, during a period when samples were taken every three hours; and the second towards the end of the experiment, when cells were probably attaining stationary growth phase. Culture 3.10d was already in the stationary growth phase when cells were harvested, and it is likely that some toxin leakage might have occurred. Extracellular OA and DTX-1 concentrations of the culture media of strain 3.10d were high, but they represented less than 0.5 % of the total toxin content.

Increased extracellular toxin concentrations of OA and DTX-1 found in some culture media could also be associated with cell debris that were suspended in solution after the harvesting of cells by centrifugation.

In this study, only OA and DTX-1 were detected in the culture media, and no DTX-4 was found, in agreement with Pan et al. (1999) findings. According to these authors, it is more likely that *P. lima* excretes DTX-4 to the media, and this compound is then enzymatically transformed to OA immediately after its extracellular release. OA and DTX-1 are probably excreted to the media in low amounts (Pan et al. 1999).

Rausch de Traubenberg and Morlaix (1995) also reported OA release to the culture media by a *P. lima* strain isolated from Vigo, Spain. These authors found that 19 to 29% of the total OA was present in the extracellular media.

Culture experiments

Growth rates

In general, results from experiment 1 were much more variable than results from experiments 2 and 3. For example, cell abundances and chlorophyll-a data from experiment 1 presented larger error bars. This may be linked to the poor physiological condition of the cells which were subjected to manual stirring every 3 or 6 days. In pelagic phytoplankton communities it is commonly observed that dinoflagellates tend to bloom after periods of water column stability and are recognized as a sensitive taxa to mixed conditions (Sullivan and Swift, 2003, Berdalet and Estrada, 1993, Estrada and Berdalet, 1998). Berdalet and Estrada (1993) have shown that dinoflagellates are particularly sensitive to water agitation in comparison with other phytoplankton groups, suggesting that mixing may affect physiological processes related to cell division. Peters and Marrase

(2000) reported a negative effect of turbulence on the growth rates of dinoflagellates, up to a bulk 121 % decrease in the normalized growth rate. These authors speculated that dinoflagellates could be physiologically impeded by turbulence. In a recent study, Sullivan and Swift (2003) have found positive, negative or insignificant effects of small scale turbulence on the growth rate of different dinoflagellate species and have proposed that the effects of small scale turbulence are likely to be a species-specific physiological factor.

Although there are no similar studies conducted using epiphytic species, it is reasonable to assume that epiphytes, that live attached to substrata, may be even more sensitive to turbulence than planktonic species. *P. lima* is weakly motile and is mostly found associated with sandy sediments and on macroalgae in moderately low or low energy environments (Tindall and Morton, 1998). The poor growth of *P. lima* (strain 2.5a) during experiment 1, when the growth rate (0.02 day^{-1}) was 5 times less than the growth rate measured during experiment 3 (strain 2.9a) was probably related to the sensitivity of this species to turbulent mixing. It is also reasonable to speculate that physiological differences between strains 2.5a and 2.9a were responsible for the different growth rates observed. Although Pan et al. (1999) have not reported any changes in *P. lima* growth rates due to turbulent stirring of cultures, they noted increased excretion/leakage of DSP toxins from *P. lima* cells exposed to intensive sampling turbulence, suggesting that this might have been caused by some kind of stress.

During experiment 2, a low growth rate was measured (0.02 day^{-1}), despite a reduction in turbulent mixing. However, the data were more consistent and less variable than in experiment 1 and a gradual increase in cell numbers was observed from day 6 to day 30. It is possible that the daylength (12 h) played a role in limiting growth.

When the light period was increased to 16 hours and cells were cultured for 40 days, during experiment 3 (strain 2.9a), the growth rate measured was 0.11 day^{-1}. This value is in the range reported for *P. lima* strains isolated from other temperate coastal regions, for instance Spain, Canada and Japan (Table 6.10). Differences in growth rate observed between experiments 2 and 3 might also be partially due to genetic differences between the two strains studied, but it is likely that growth conditions like the increased light period of 16 hours played a major role in determining the higher growth rate attained during experiment 3.

Table 6.10: Growth rates (day^{-1}) of different strains of *P. lima* isolated from various locations.

Strain, place of origin	Growth rate	Reference	Comments
PL11V, Ria de Vigo, Spain	0.06	Bravo et al. 2001	Natural seawater, K media, 19 °C, 60-70 μmol $m^{-2}s^{-1}$, 14L:10D. Measurements considered only two points on the growth curve, on days 13 and 27.
PL27V, Ria de Vigo, Spain	0.08	Bravo et al. 2001	Natural seawater, K media, 19 °C, 60-70 μmol $m^{-2}s^{-1}$, 14L:10D. Measurements considered only two points on the growth curve, on days 13 and 27.
PL2V, Ria de Vigo, Spain	0.13	Bravo et al. 2001	Natural seawater, K media, 19 °C, 60-70 μmol $m^{-2}s^{-1}$, 14L:10D. Measurements considered only two points on the growth curve, on days 13 and 27.
PL6V, Ria de Vigo, Spain	0.14	Bravo et al. 2001	Natural seawater, K media, 19 °C, 60-70 μmol $m^{-2}s^{-1}$, 14L:10D. Measurements considered only two points on the growth curve, on days 13 and 27.
PL2V, Ria de Vigo, Spain	0.092	Morlaix and Lassus (1992)	Natural seawater, K media, 20 °C, 24 μmol $m^{-2}s^{-1}$, 12L:12D
Pa, Nova Scotia, Canada	0.11 – 0.12	Pan et al. (1999)	Natural seawater, L1 media, 18 °C, 90 μmol $m^{-2}s^{-1}$, 14L:10D
Strain from Dry Tortugas, Florida	0.75	Tomas and Baden (1993)	Natural seawater, K media, 26 °C, 150 μmol $m^{-2}s^{-1}$, 16L:8D
PL100A, Knight Key, Florida	0.47	Morton and Norris (1990)	Natural seawater, K media, 27 °C, 180 μmol $m^{-2}s^{-1}$, 14L:10D
A249, Heron island, Australia	0.35	Morton and Tindall, (1995)	Natural seawater, K media, 28 °C, 30-50μmol $m^{-2}s^{-1}$, 16L:8D
A18, Heron island, Australia	0.20	Morton and Tindall, (1995)	Natural seawater, K media, 28 °C, 30-50μmol $m^{-2}s^{-1}$, 16L:8D
Strain from Sanriku, Japan	> than 0.20	Koike et al. (1998)	T1 media, 15, 20 or 25 °C, 170 μmol $m^{-2}s^{-1}$, 14L:10D
P6, from New Caledonia	0.27±0.06	Holmes et al. (2001)	F10k media, 25-29 °C, 50-90 μmol $m^{-2}s^{-1}$, 12L:12D
Strain PRL-1, Baja California Sur, Mexico	0.107±0.003	Heredia-Tapia et al. (2002)	ESI media, 22 °C, 4 x 20W fluorescent lamps, 12L:12D
Strain 2.9a, Fleet lagoon, UK	0.11	This study	Artificial seawater, L-2 media, 17 °C, 90 μmol $m^{-2}s^{-1}$, 16L:8D

Nitrate and phosphate consumption from growth media

During experiment 3, nitrate and phosphate concentrations in the growth media became undetectable between days 17 and 25. Luxury phosphate consumption by phytoplankton, when transport is greater than that required to match steady-state demand is considered to be an extremely common phenomenon (Fogg and Thake, 1987). The implications of having enhanced nutrient transport capabilities are important in resource competition and can counter suboptimal internal kinetics of resource use (Flynn, 2002). In experiment 3, cell numbers continued to increase after nitrate and phosphate concentrations in the media were less than 1 μM. Cell numbers increased from 30542 cells mL^{-1} on day 25 to 48308 cells mL^{-1} on day 45. Although cells were not growing

exponentially during this time, the increase in cell numbers indicates that *P. lima* has used the intracellular nutrients to continue cellular division.

Pan et al. (1999) reported *P. lima* ammonia and nitrate uptake rates of 6.04 and 6.12 pmol $cell^{-1}$ day^{-1}. In their experiment, nitrate uptake was initiated only after the initial 65 μM of ammonia was exhausted and only in the presence of light. Also in their experiment, *P. lima* was able to assimilate nearly 500 μM of nitrate and around 23 μM of phosphate, both being exhausted from the culture media during a period of 18 days under a 14 hours light, 10 hours dark cycle. These authors reported an increase in nitrite concentrations (up to 15 μM) soon after nitrate levels started to decrease in the media, followed by a rise in ammonia levels. They suggest that this is probably the result of excess nitrite and ammonia produced by nitrate reduction and subsequent efflux from the cells (Pan et al. 1999). The method used here to quantify nitrate in the growth media does not differentiate between nitrate and nitrite, and measures the sum of $NO_3 + NO_2$. The release of nitrite by *P. lima* cells would explain the occasions when an increase in nitrate+nitrite concentrations were observed, as from day 25 to 31 of the 3rd experiment and days 18 to 24 of the 1st experiment.

The exhaustion of nutrients in the growth media by *P. lima* contrasts with the results obtained for *A. minutum* (chapter 5), that ceased growth when concentrations of nitrate and phosphate in the growth media were 615 μM and 6.0 μM respectively. Moreover, other authors that have studied *A. minutum* (Flynn et al. 1994) and *A. tamarense* (Anderson et al. 1990) have come to the conclusion that dinoflagellates often cease growth when there are still excess amounts of macronutrients present in the media, and that this may be due to dissolved inorganic carbon stress, especially under high cell densities. The ability to use HCO_3^- indirectly through the catalytic production of CO_2 by extracellular carbonic anhydrase activity under conditions of CO_2 limitation was already demonstrated for another species of *Prorocentrum*, *P. micans* (Nimer et al. 1999). It is possible that *P. lima* also possess a similar mechanism that allows this species to continue growth under conditions of dissolved inorganic carbon stress.

During experiment 3, the N:P draw down ratio was 16.5:1, consistent with the N:P Redfield ratio of 16:1 for particulate matter in the sea. The nitrate to phosphate draw-down ratio of experiment 2 (4.0) is lower than the N:P Redfield ratio. However, Geider and La Roche (2002) have shown that inorganic N:P draw-down ratios can range from 4.4 to 19 and that values lower then 16:1 are associated with nutrient replete-cells with an intracellular accumulation of phosphate.

Pigment composition

In addition to chl-a, *P. lima* 2.5a and 2.9a contained minor amounts of chl-c_1+c_2 (not separated by the present HPLC method) and chl-c_3. Peridinin was the major carotenoid in *P. lima*. Other carotenoids detected were diadinoxanthin, diatoxanthin, β-carotene and 19'- butanoyloxyfucoxanthin. Chl-c_2 has been reported to be a major pigment in other species of dinoflagellates, such as *Karenia mikimotoi* (as *Gymnodinium mikimotoi*) (Hansen et al. 2000) and *Amphidinium carterae* (Wright et al. 1991), but was only a minor pigment in *P. lima* strains 2.5a and 2.9a. Morton and Tindall (1995) reported concentrations of chl-c_2 that varied between 0 and 9 pg cell^{-1} in 17 clones of *P. lima* isolated from Heron Island, Australia. Other *P. lima* pigments described by these authors were chl-a, peridinin and other xanthophylls (dinoxanthin and diatoxanthin), that were not separated by the thin layer chromatography (TLC) method they employed. In *Prorocentrum hoffmannianum*, chl-c_2 concentrations varied between 4 and 10 pg cell^{-1} in a batch culture and elevated maximum concentrations of chl-a (up to 65 pg cell^{-1}) and peridinin (up to 40 pg cell^{-1}) were reported (Aikman et al. 1993).

Chl-c_3 has been reported to occur in only one other dinoflagellate species, *Karenia mikimotoi* (as *G. mikimotoi*) (Hansen et al. 2000), also as a minor component. Chl-c_3 is normally only found in some prymnesiophytes, some diatoms and chrysophytes (Jeffrey and Vesk, 1997).

19'- butanoyloxyfucoxanthin has been reported in dinoflagellates that possess an anomalously pigmented plastid, in which the carotenoid composition is dominated by fucoxanthin, 19'-hexanoyloxy- and 19'-butanoyloxy-fucoxanthin, in spite of peridinin, the typical carotenoid present in dinoflagellates. Examples of species of this group of dinoflagellates are *Karenia mikimotoi* (as *Gymnodinium mikimotoi*) (Hansen et al. 2000), *Gymnodinium galatheanum*, *Gyrodinium aureolum* (probably =*Karenia mikimotoi*) and *Karenia brevis* (as *Gymnodinium breve*) (Tengs et al. 2000).

A peak at the retention time of 19'- butanoyloxyfucoxanthin and with similar absorption spectra was found in extracts of *P. lima* strains 2.5a and 2.9a, and was identified as this pigment. Concentrations were low however.

Chloroplasts are believed to have an endosymbiotic origin (Palmer 2003). Within the dinoflagellates there are several different plastid types that are thought to be the result of secondary endosymbiosis events. The plastids of peridinin-containing dinoflagellates are related to red algal plastids (Tengs et al. 2000). A second group including *Dinophysis* species have plastids containing phycobilins and chl-a and c (Hewes et al. 1998). A third group comprises fucoxanthin containing dinoflagellates, that are though to have acquired

their plastids via endosymbiosis of a haptophyte, and are the result of three sequential endosymbiotic events (Tengs et al. 2000). Interesting cases of serial plastid symbiosis like tertiary symbiosis and secondary replacement, in which a red algal derived plastid is replaced by a plastid of green algae occurs in dinoflagellates and represent intriguing cases (Palmer, 2003).

The chloroplast envelope of *P. lima* is typical of other dinoflagellates and is composed of three membranes (Zhou and Fritz, 1994). Most plastids surrounded by more than two membranes are though to be the result of secondary endosymbiosis events (Bhattacharya and Medlin, 1995, Tengs et al. 2000). The production of 19'buta by *P. lima* and its possible implications needs to be further investigated.

Jeffrey et al. (1975) reported the pigment composition of two species of *Exuviella*, *E. sp.* and *E. cassubica*, that is now a synonym for *Prorocentrum cassubicum*, a sand-dwelling species (Dodge and Lewis, 1986). This species showed a similar pigment profile to the one reported here for *P. lima*, except that they have reported the presence of dinoxanthin, that was not found in *P. lima* in the present study. Jeffrey et al. (1975) also reported the presence of an unknown pigment in the *P. cassubicum* chromatogram, that could have been 19'- butanoyloxyfucoxanthin.

Light harvesting pigments, like chl-a, peridinin, diadinoxanthin and chl-c_2 are expected to covary, peaking during exponential growth phase and then decreasing towards stationary phase. This pattern was observed during experiment 3, when chl-a and peridinin peaked during mid exponential growth phase on day 17, then decreased until day 31, and then were constant until day 45.

β-carotene, diadinoxanthin and diatoxanthin are suggested to have a photoprotective role, the later two via the xanthophyll cycle. Xanthophylls are oxygenated derivatives of the carotenes (Bjornland, 1997). The xanthophyll cycle of higher plants, green and brown algae involves the conversion of zeaxanthin to antheraxanthin and the later to violaxanthin through epoxidation of zeaxanthin (Porra et al. 1997 and references therein). A two component xanthophyll cycle, involving conversion of diadinoxanthin to the epoxy-free diatoxanthin is present in all chromophyte algal groups. In dark adapted cells, most of the xanthophyll-cycle pigments are in the epoxide form (diadinoxanthin). De-epoxydation *in vivo* is triggered by high light, or more generally, when light absorption exceeds light utilization by photosynthesis (Porra et al. 1997), to protect the photosynthetic apparatus against photoinhibition. Demers et al. (1991) observed a fast and reversible increase in the concentration of diatoxanthin, at the expense of its parental compound, diadinoxanthin, in *Alexandrium excavatum* upon exposure to high light. Data

presented here suggest that diadinoxanthin was gradually converted into diatoxanthin as the culture developed in experiment 3. Diadinoxanthin concentrations decreased from 6.7 pg $cell^{-1}$ on day 17 to 3.0 pg $cell^{-1}$ on day 45 while diatoxanthin increased from 1.0 pg $cell^{-1}$ to 3.9 pg $cell^{-1}$ during the same period. The incubator light levels were not changed during the experiment and it is likely that as the cultures became more dense, less light was available due to self shading. This suggests that other factors may be involved in the conversion of diadinoxanthin to diatoxanthin, possibly processes related to the senescence of the culture. It is also possible that "de novo" synthesis of diatoxanthin occurred.

OA and DTX-1 toxins

OA and DTX-1 per cell decreased initially in experiment 3, remaining quite constant during the exponential growth phase, and then increased towards the stationary growth phase, when cell division had decreased. The elevated cellular content during this phase suggests that the rate of toxin synthesis remained constant throughout the experiment but toxins accumulated in the cell when the cell division rate decreased. Maximum toxin content of *P. lima* (McLachlan et al. 1994, Quilliam et al. 1996, Bravo et al. 2001, Holmes et al. 2001) and *P. hoffmannianum* (Aikman et al. 1993) in the stationary phase has previously been described by other authors. A decline in toxin content of *P. lima* cells as the culture entered exponential phase from the initial lag phase was also observed by Jackson et al. (1993), however, these authors terminated their experiment when cells were still growing exponentially.

Toxin composition did not vary appreciably during the growth of strains 2.5a and 2.9a, although DTX-1 concentrations increased at a slightly higher rate towards the stationary phase, as indicated by the slight decrease in the OA/DTX-1 ratio.

DSP toxins occur in widely divergent taxa, such as in the sponge *Halichondria* (possibly as a product of an endosymbiotic dinoflagellate) and in the dinoflagellates *Prorocentrum* and *Dinophysis*. This gives an indication of possible polyphyletic and/or endosymbiotic origin of these compounds (Wright and Cembella, 1998). OA has been shown to be localized mainly in the chloroplasts and pyrenoids of *P. lima* (Zhou and Fritz, 1994). It is interesting to note that some dinoflagellate genera like *Dinophysis* and *Gymnodinium* that produce polyether toxins (DSP toxins, brevetoxins, gymnodimine) have anomalously pigmented plastids. DSP toxins are produced by epiphytic *Prorocentrum* and planktonic *Dinophysis*, and remarkably all toxigenic *Dinophysis* are known to be autotrophic. The few studies that have reported pigment composition of epi-bethic *Prorocentrum* species have shown that peridinin is the major carotenoid in the

species studied, however the presence of 19'buta in the *P. lima* strains 2.5a and 2.9a suggests that *P. lima* pigment composition should be further investigated using HPLC techniques in other strains. *Prorocentrum* and *Dinophysis* are considered to be primitive and close phylogenetic relatives, based primarily upon their bilateral symmetry, apical inserted flagellae and thick-walled theca (Taylor, 1987). However, these genera differ widely in their ecological niche, which begs the question of whether the expression of the common trait of toxin production merely represents a shared evolutionary artifact or if it is integral to ensuring survival in diverse habitats (Wright and Cembella, 1998). DSP toxins are thought to have an allelopathic role based on their phosphatase inhibiting activity. However, if toxin production is a shared evolutionary artifact, it is possible that there exists some relationship between the mechanisms that have lead to anomalously pigmented plastids in dinoflagellates and the capability to produce different types of polyether toxins. This idea is totally speculative, and further studies on pigment composition and plastid structure of polyether toxin producing dinoflagellates are necessary.

6.4 Chapter summary

Data presented in this chapter indicates that *P. lima* is the most likely source of DSP toxins in shellfish from the Fleet lagoon. All 20 *P. lima* strains isolated from the Fleet were shown to produce both OA and DTX-1. Toxin composition clearly varied depending on the strain as well as total toxin production, which ranged from 0.42 to 17.13 pg OA $cell^{-1}$ and 0.41 to 11.29 pg DTX-1 $cell^{-1}$. A number of diol ester derivatives of OA and DTX-4 were also identified in *P. lima* strains from the Fleet. *Dinophysis* cells were not detected in plankton and net samples collected from the lagoon during 2000 and 2001 and were not previously detected in the Fleet by the CEFAS plankton monitoring program (S. Milligan, pers. comm.), which also indicates that *P. lima* is the source of DSP toxins detected in shellfish from the lagoon during summer 2000.

Most of the OA and DTX-1 toxins were present inside *P. lima* cells and were not released to the culture media. Considering the 20 *P. lima* strains screened, more than 95% of OA or DTX-1 concentrations were measured inside the cells in 17 strains. In the other three strains, a higher amount (up to 67%) of toxins was released to the culture media, which could have been caused by mixing and detachment of *P. lima* cells from the bottom of the culture flask or leakage of toxins from senescent cells.

Culture experiments using strains 2.5a and 2.9a revealed that *P. lima* is highly sensitive to turbulent mixing during culture sub-sampling and therefore the species had to

be grown in different culture flasks. Differences were observed in the growth rate, OA and DTX-1 absolute concentrations of strains 2.5a and 2.9a during the growth experiments, although it is recognized that they were not maintained under the same length of photoperiod in experiment 2 (strain 2.5a) and experiment 3 (strain 2.9a). However, the amounts of OA and DTX-1 produced by these two strains during the growth experiments were consistent with the initial OA and DTX-1 screening of all 20 strains. Maximum OA and DTX-1 content of *P. lima* was observed at the stationary growth phase, suggesting that the rate of toxin synthesis remained constant throughout the experiment, but toxins accumulated in the cell when the cell division rate decreased. These results are in agreement with the findings of other authors who have studied *P. lima* and *P. hoffmannianum*.

Peridinin was the major carotenoid in *P. lima* strains 2.5a and 2.9a. Chl-a, diadinoxanthin, diatoxanthin, β-carotene as well as minor amounts of chl-c_1+c_2, chl-c_3 and 19'buta were also detected. The production of the latter pigment by these two strains of *P. lima* deserves further investigation of other *P. lima* isolates using HPLC methods.

During experiment 3 (strain 2.9a), nitrate and phosphate concentrations in the growth media became undetectable between days 17 and 25 and cell numbers continued to increase until day 45. Although cells were not growing exponentially from day 25 to 45, the increase in cell numbers indicates that *P. lima* was able to continue dividing using the accumulated nutrients when these were exhausted from the culture media.

Chapter 7

Conclusions and main findings of this research

1. The region of the Abbotsbury embayment in the Fleet lagoon can be considered eutrophic based on the levels of nutrients, annual mean chl-a concentration and phytoplankton biomass results from the two year survey. This is in contrast to the eastern part of the Fleet lagoon, that presents lower values of these parameters. The eutrophication of the Abbotsbury embayment is caused by high nutrient inputs to this area together with the weak flushing of the west Fleet. Phytoplankton are important primary producers in the Fleet lagoon from April to November but only in the west Fleet, particularly at Abbotsbury in spring and late summer. In contrast, in the mid and east Fleet, low chlorophyll-a values during summer is an indication that macroalgae and eelgrass are outcompeting phytoplankton during this period. Therefore, nutrient conditions are not likely to be limiting phytoplankton growth at the Abbotsbury embayment, except for periods following intensive growth (bloom conditions). Nitrogen limitation is likely to take place at stations 2 to 7 from spring to autumn, and phosphorus is not likely to be limiting.

2. Blooms of cryptophytes that occur during the spring at Abbotsbury are probably caused by rich nutrient conditions due to winter input through rainfall and runoff and the capability of these small flagellates to grow rapidly and acclimate to light conditions that would be limiting for other phytoplankton species.

3. The balance between freshwater and marine inputs to the west Fleet lagoon is an important determinant of *Prorocentrum micans* blooms at the west Fleet. During 2000, marine waters could move further into the Fleet lagoon, due to lower freshwater inputs, transporting *P. micans* cells to the area of suitable growth conditions of high nutrient stocks (station 1 and 2) during late July-August, when peak temperatures are recorded and seaweeds and eelgrass are dieing off. The combined effects of favorable growth conditions of nutrients, temperature and longer water retention time determined the *P. micans* bloom in 2000. The confinement of the water and the minimal loss through advection created suitable conditions for *P. micans* to develop high biomass. In contrast, during 2001, due to the increased freshwater input to the west Fleet, marine waters did not penetrate much into the lagoon, and *P. micans* did not reach the favorable conditions of stations 1 and 2 at all during 2001. A bloom represents normal population increases that are confined, and is not caused by sudden population explosions due to increased division rates. Nutrient enrichment of the west end of the Fleet increases the carrying capacity of the system and the potential magnitude of blooms of cryptophytes and dinoflagellates, but in itself, does not trigger their development.

4. *Prorocentrum lima* is the most likely source of DSP toxins in shellfish from the Fleet lagoon. All 20 *P. lima* strains isolated from the Fleet were shown to produce both OA and DTX-1. Toxin composition and total toxin production clearly varied depending on the strain. A number of diol ester derivatives of OA and DTX-4 were also identified in *P. lima* strains from the Fleet. Other DSP producers such as *Dinophysis* spp. were not detected in plankton and net samples collected from the lagoon during 2000 and 2001, which also indicates that *P. lima* is the source of DSP toxins detected in shellfish from the lagoon during summer 2000. Maximum OA and DTX-1 content of *P. lima* was observed at the stationary growth phase, suggesting that the rate of toxin synthesis remained constant throughout the experiment, but toxins accumulated in the cell when the cell division rate decreased. *P. lima* exhibited enhanced nutrient assimilation capabilities, that are important in resource competition, and cells were able to continue growing using their internal nitrogen and phosphate when these nutrients were exhausted from the culture media. All strains studied presented typical morphological characteristics of the species and some cells showed relatively straight sides. The pigment profile

of the two *P. lima* strains studied were typical of peridinin-containing dinoflagellates, but the presence of low amounts of 19'buta is unusual and deserves further investigation in other *P. lima* isolates using HPLC methods.

5. *Alexandrium minutum* strain 3.9h isolated from the Fleet has a unique PSP toxin profile containing GTX-2 (9.6 mole %), GTX-3 (54.3 mole %) and STX (36.1 mole %).The toxin composition of this *A. minutum* strain is quite different to that reported for other isolates of this species from sites throughout the world. Maximum cellular toxicity of 4.8 pg STX equiv $cell^{-1}$ was attained during early stationary phase and after this point toxicity began to decrease. Previous studies have demonstrated that the *A. tamarense* population from the southwest of England is non toxic. The description of STX production by *A. minutum* from the south coast of England indicates that this species may be the source of PSP toxins found in shellfish in this region. The presence of a ventral pore on the suture between the first and fourth apical plates was variable in this strain and therefore should not be used to identify this species. *A. minutum* 3.9h ceased growth when concentrations of nitrate and phosphate in the growth medium were 615 μM and 6.0 μM and this may be due to dissolved inorganic carbon stress, especially under high cell densities. The pigment profile of *A. minutum* 3.9h was typical of peridinin-containing dinoflagellates, including chl-a, peridin, diadinoxanthin, chlorophyll-c_1+c_2 and c_3, diatoxanthin, and β-carotene.

6. The HPLC pigment composition of samples from the Fleet lagoon was in general a good descriptor of changes observed in the phytoplankton community of the lagoon. The blooms of cryptophytes and of *P. micans* could be identified using pigment data, as well as the minor contribution of diatoms to the total phytoplanktonic biomass and on a few occasions dominated by chl-b containing organisms (euglenophytes). However, microscopy revealed changes in cell abundance and group specific species composition, which is highly valuable information for ecological research that can not be obtained by pigment analysis. This latter technique proved to be particularly useful to assess the taxonomic composition of nanoflagellates, that could not be identified by microscopic analysis, as its taxonomy is poorly known, and their trophic status can not be determined using ordinary light microscopy. However, some inconsistencies between the two techniques were observed as on two occasions in which the

presence of peridinin and fucoxanthin were not correlated with significant abundances of dinoflagellates and diatoms and on another day a high biomass of *E. marina* was recorded by microscopic analysis, but no associated chl-b was detected by HPLC.

7.1 Future work and recommendations

The results of this thesis show that the Abbotsbury embayment in the Fleet lagoon can be considered eutrophic. However, due to the absence of previous phytoplankton and water quality data from the Fleet lagoon it is difficult to say if current conditions were caused by recent increased anthropogenic inputs to this region of the lagoon. Therefore it is important to continue monitoring the water quality and phytoplankton species and abundance in the Fleet, particularly at Abbotsbury to establish a temporal dataset. This dataset will also help better understanding the conditions triggering blooms of different phytoplankton species, and will be helpful in clarifying the factors responsible for the interannual variability observed in the lagoon. The input of freshwater to the Fleet seems to be important both as a source of nutrient and as a physical boundary and it would be desirable to quantify the major freshwater inputs to the lagoon, as river flow and runoff. Other variables that were not included in this research and that are likely to be important in determining the occurrence of phytoplankton blooms in the Fleet are the dissolved organic nitrogen and phosphorus pool. It would be important to determine using laboratory cultures if species that bloom in the lagoon are able to utilize dissolved organic nutrient pools.

Data from winter conditions in the Fleet lagoon are very scarce and this season should be included in a future monitoring program to address if diatoms are more abundant along the lagoon during this season.

A more detailed taxonomic study of the phytoplankton of the lagoon would be a significant expansion of this study, particularly the small (less than 10 μm) species, including the identification of the cryptophytes and nanoflagellates. It would be important to identify the athecate dinoflagellate species that are abundant during the summer, and to assess their trophic status. The zooplankton community of the lagoon also deserves more attention, and its characterization would improve our understanding of factors related to bloom control and decline.

The quantification of the epiphytic biomass of *Prorocentrum lima*, the most probable source of DSP toxins in the Fleet should also be included in a monitoring

program aiming at an early warning of shellfish toxicity. The study of the factors causing detachment of *P. lima* cells from eelgrass and seaweeds, e.g. wind intensity, wind direction, tidal currents, rainfall, would greatly improve the understanding of the circumstances leading to the availability of *P. lima* cells to shellfish filtration and the consequent accumulation of DSP toxins in shellfish tissues.

Concerning the culture work, there are various experimentation opportunities. The influence of different environmental conditions on the toxicity and growth rate of *Prorocentrum lima* and *Alexandrium minutum* could be tested and compared with data from the literature for strains of these species isolated from elsewhere. The 20 strains of *P. lima* from the Fleet lagoon provide an opportunity to compare the genetics of the *P. lima* population of this lagoon and this results could be related to differences observed in the amount of toxin produced and the toxin profile of each strain. *A. minutum* is particularly interesting in this respect, as the analysis of the D1-D2 domain of the large subunit ribosomal DNA (LSU rDNA) of this strain (performed by E. Lilly at WHOI and included in a paper submitted for publication, Nascimento et al. 2003) showed that this strain is identical to other non-toxic *A. minutum* strain isolated from Italy. Toxicity is though to be genetically determined in *Alexandrium* species, and it would be interesting to compare this strain with the non toxic *A. minutum* strain that is genetically identical to this one and to investigate if environmental factors could switch the ability to produce PSP toxins on and off in *A. minutum*, or if a more detailed genetic study is necessary to characterize different strains of this genus.

Finally, this research has shown that there are DSP and PSP producers in the Fleet lagoon. Although they currently do not cause major shellfish contamination problems, experience from other areas have shown that as eutrophication progresses, blooms of toxic species tend to increase in intensity and incidence. Moreover, blooms of toxic species can injure and kill marine life, and may become a threat to the local wildlife of the Fleet lagoon. The confined nature of the west Fleet provides a perfect environment for these species. Management strategies that include the control of nutrient inputs to the west Fleet and the improvement of water circulation in this region would be beneficial in the long run to avoid that harmful and toxic algal blooms becoming a local problem.

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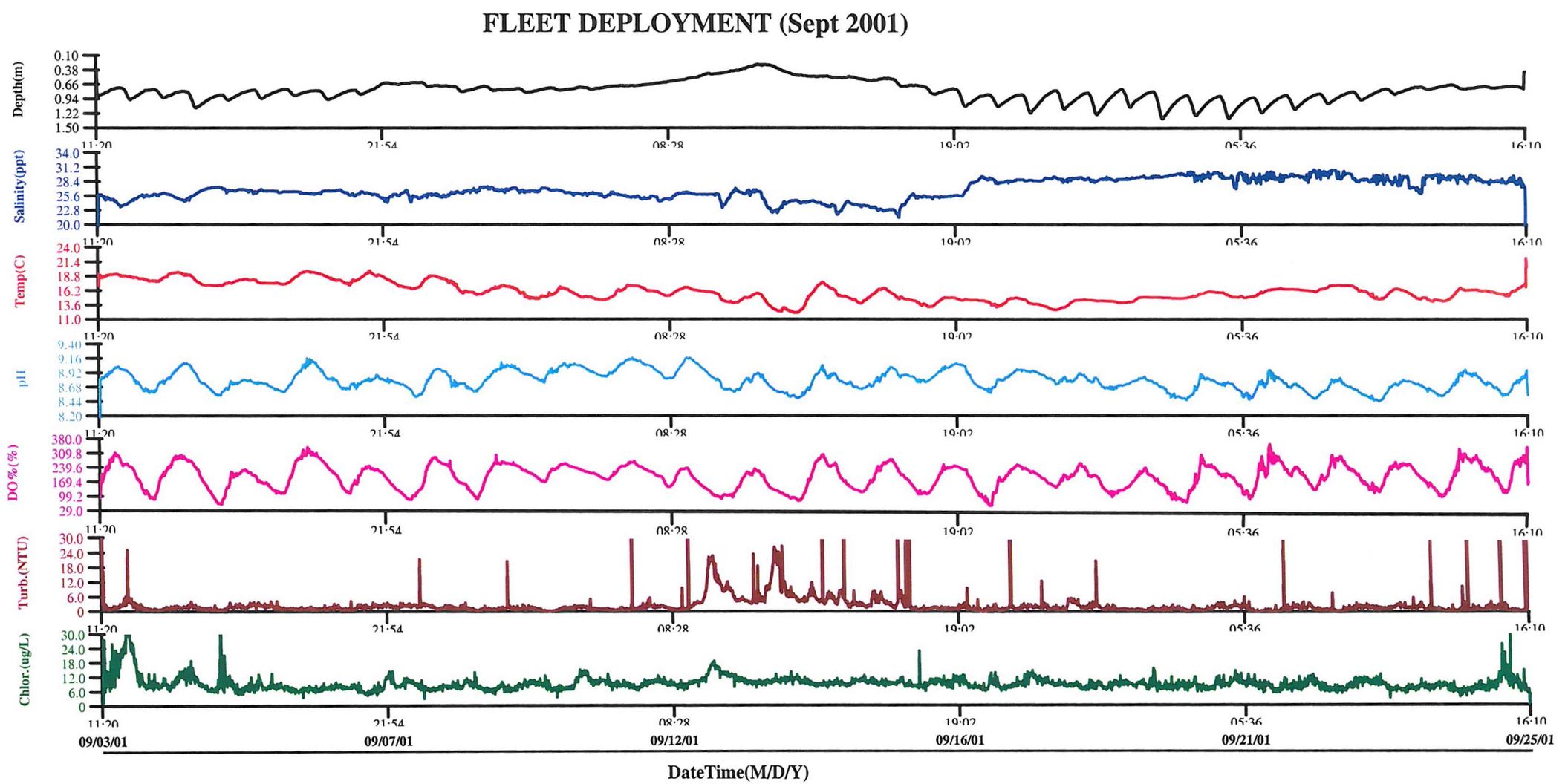
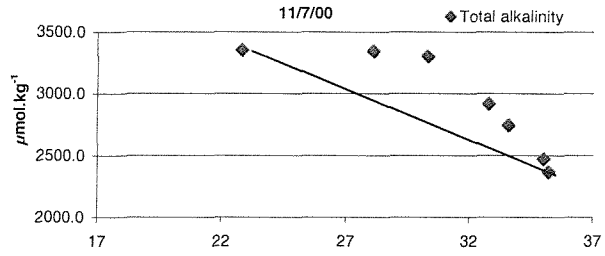
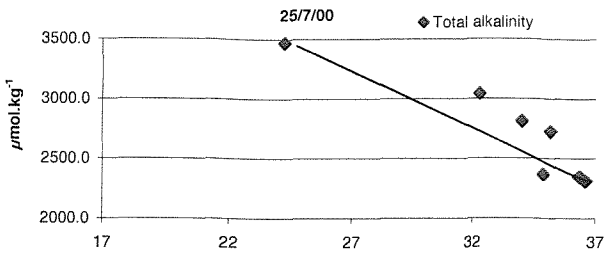
Appendix 1: Data from the YSI 6000 probe deployment at Abbotsbury from 3-25 September 2001 to measure chlorophyll-a, temperature, salinity, pH, turbidity, dissolved oxygen and depth continuously during this period.

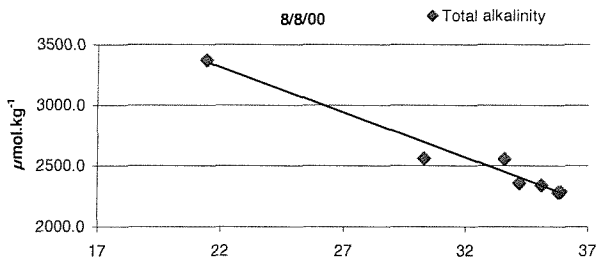


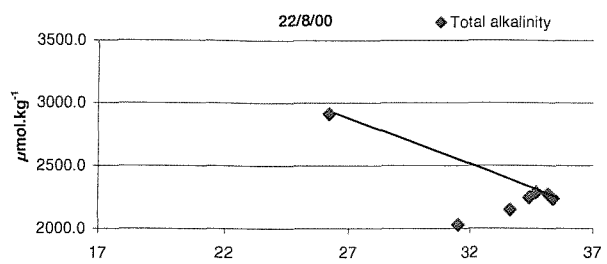
APPENDIX 2

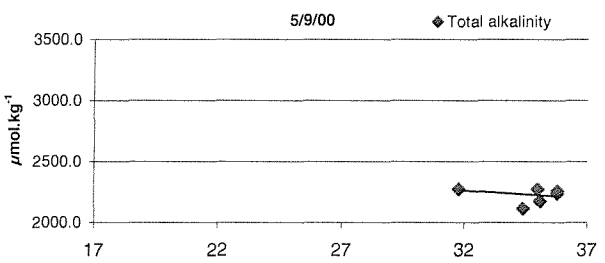
Alkalinity (μmol kg^{-1}) versus salinity plots for each day of sampling throughout the Fleet lagoon in 2000.
Lines connect data from station 1 and 7, except on the 4/11/00, when the line connects data of station 2 and 7

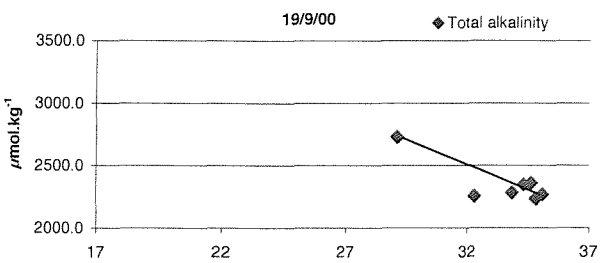


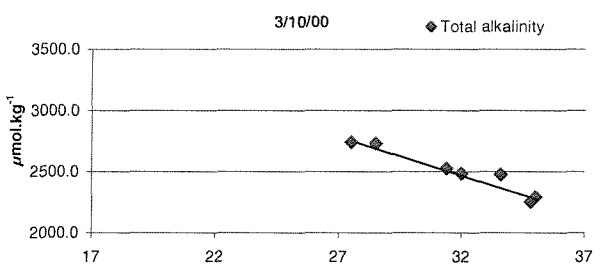


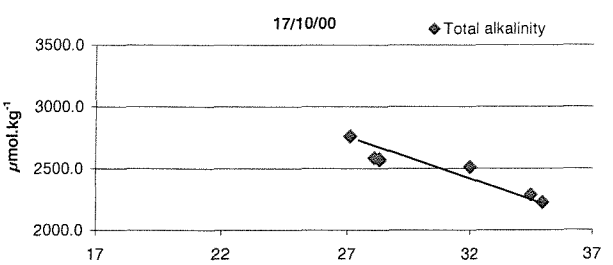


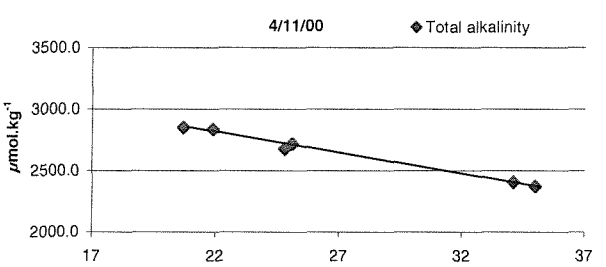






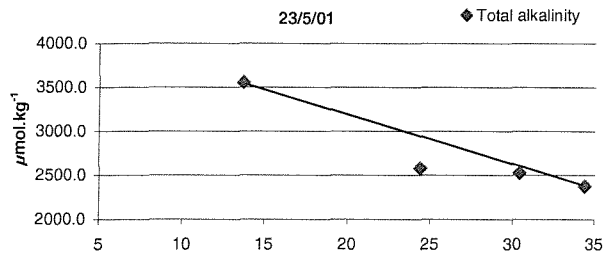


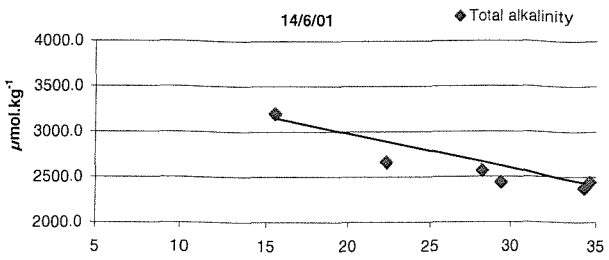


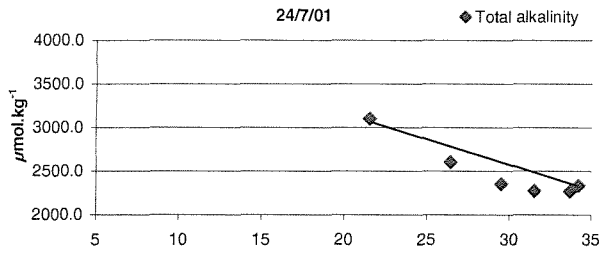


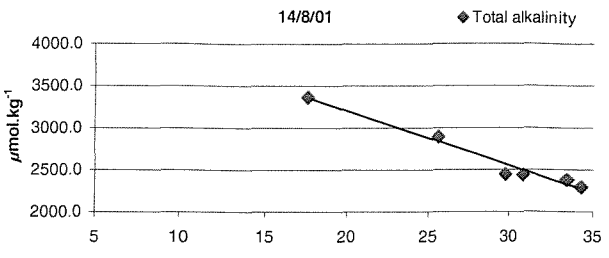
APPENDIX 3

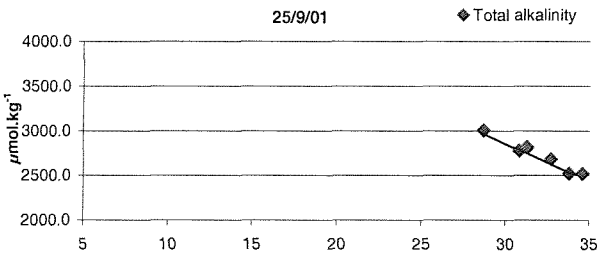
Alkalinity (μmol kg^{-1}) versus salinity plots for each day of sampling throughout the Fleet lagoon in 2001.
Lines connect data from station 1 and 6

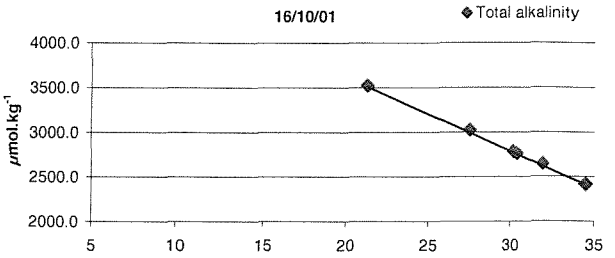


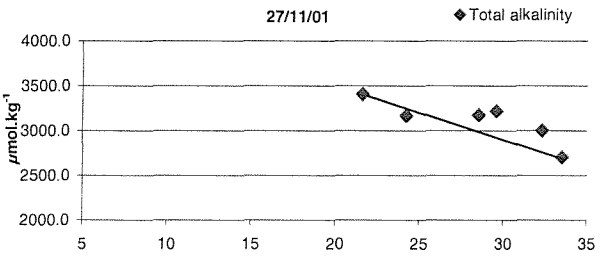






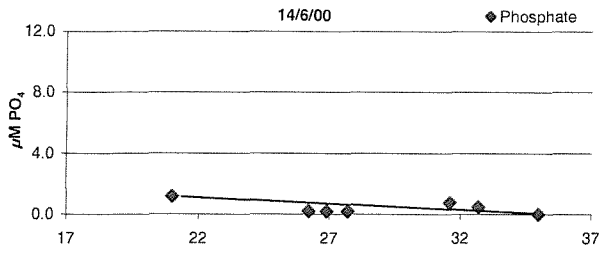


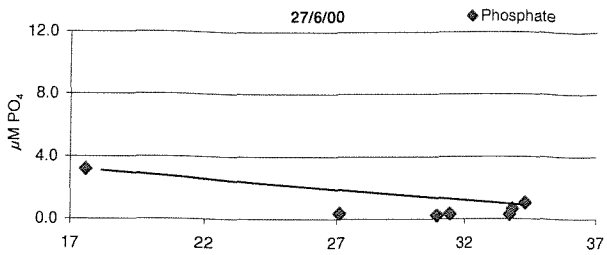


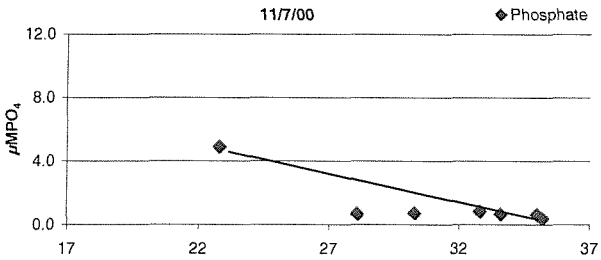


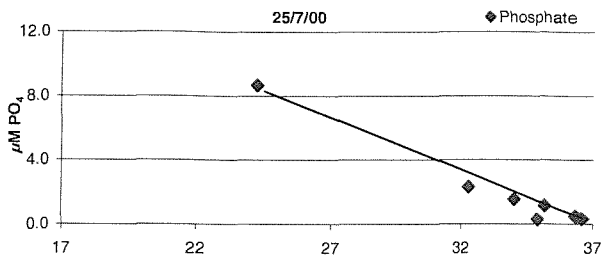
APPENDIX 4

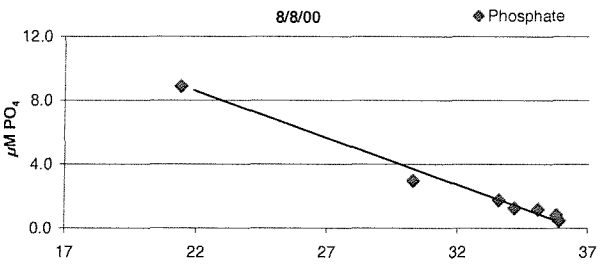
Phosphate (μM) versus salinity plots for each day of sampling throughout the Fleet lagoon in 2000.
Lines connect data from station 1 and 7

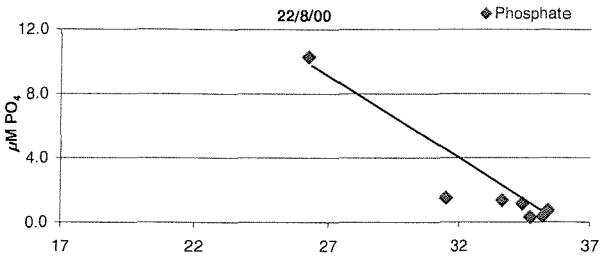


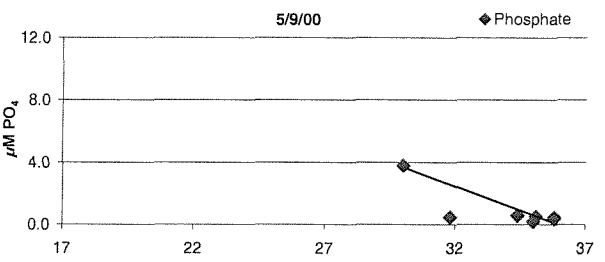


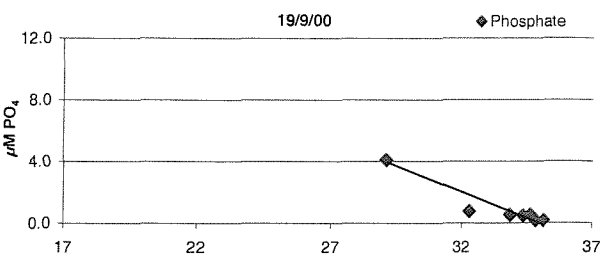


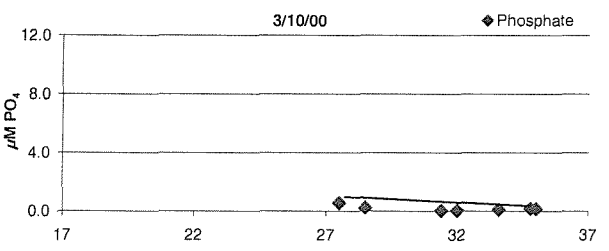


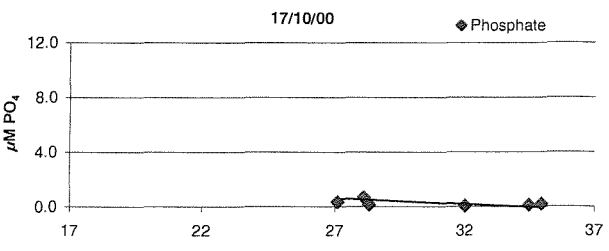


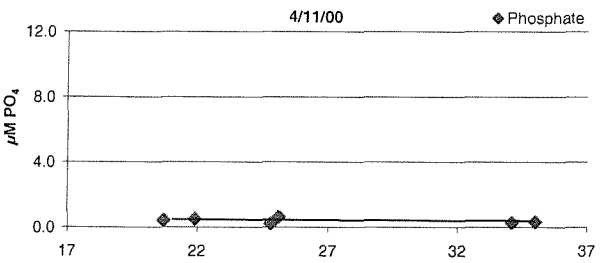






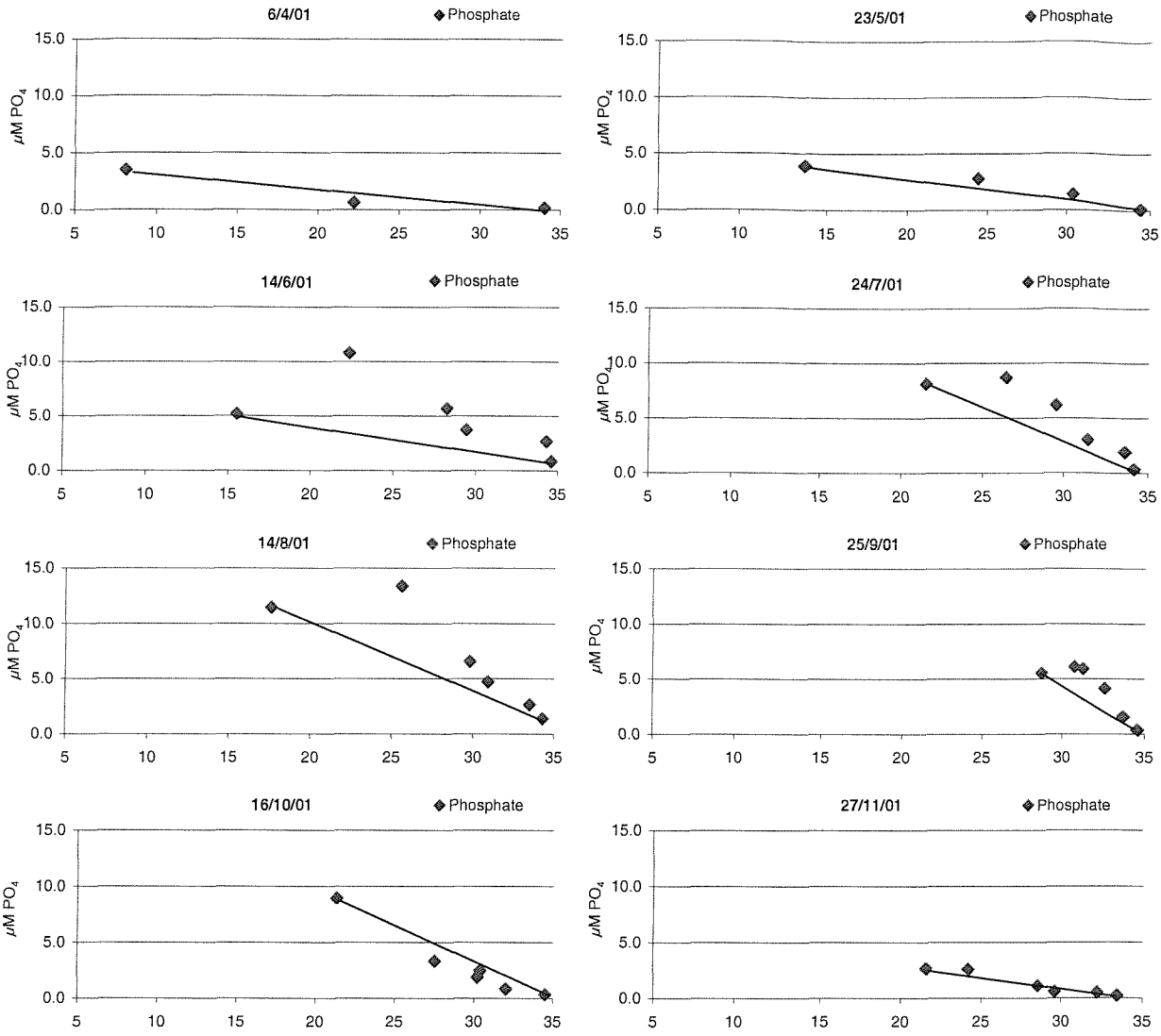






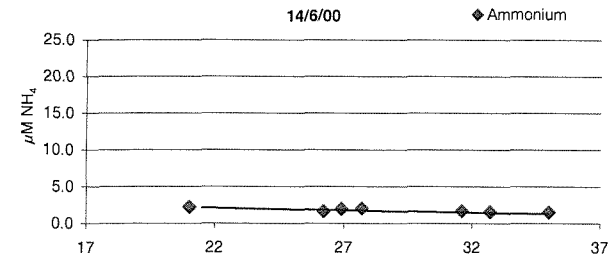
APPENDIX 5

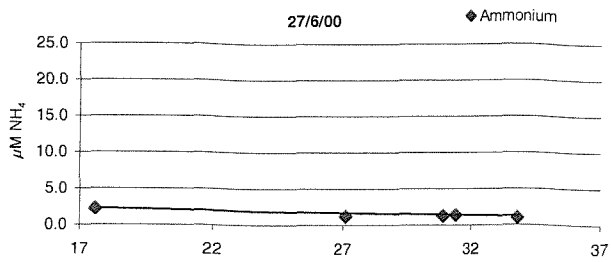
Phosphate (μM) versus salinity plots for each day of sampling throughout the Fleet lagoon in 2001.
Lines connect data from station 1 and 6

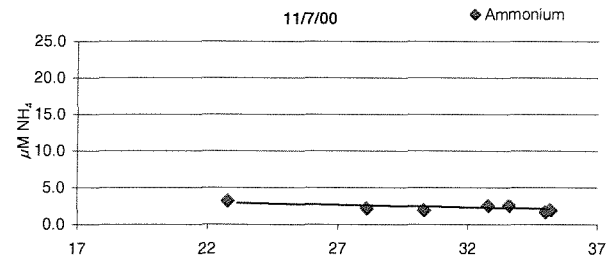


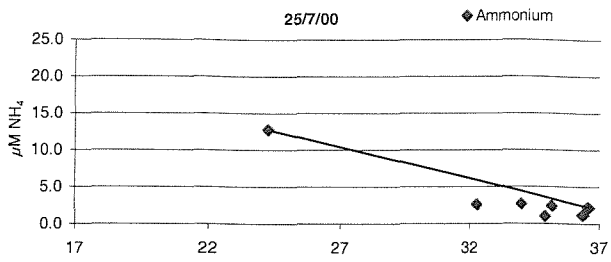
APPENDIX 6

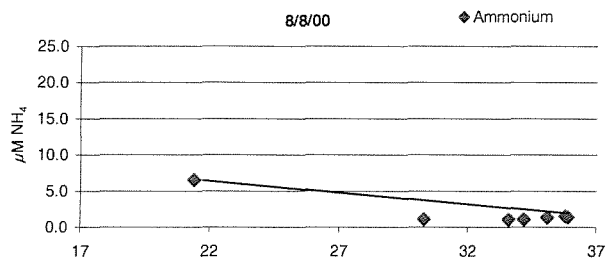
Ammonium (μM) versus salinity plots for each day of sampling troughout the Fleet lagoon in 2000
Lines connect data from station 1 and 7

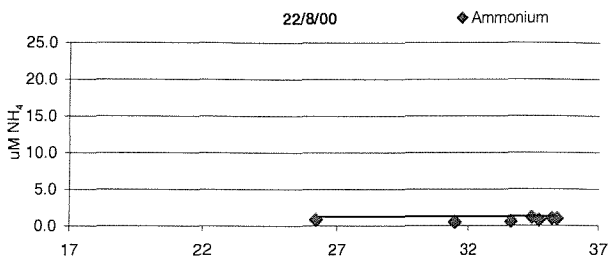


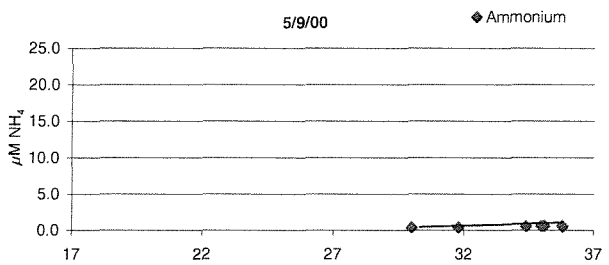


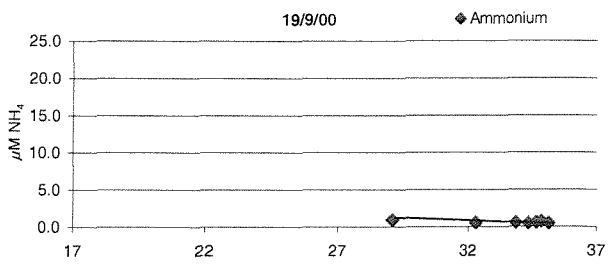


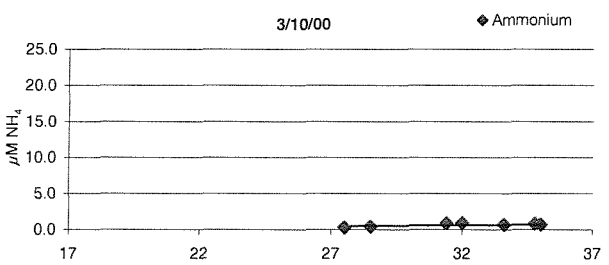


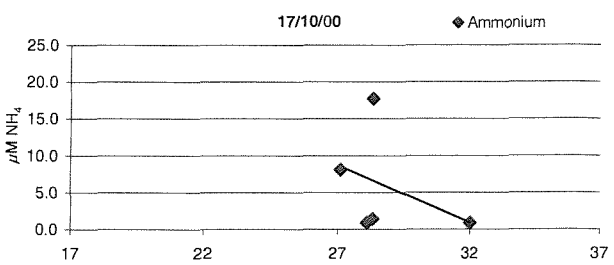


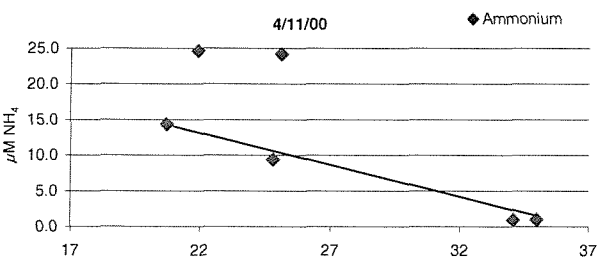






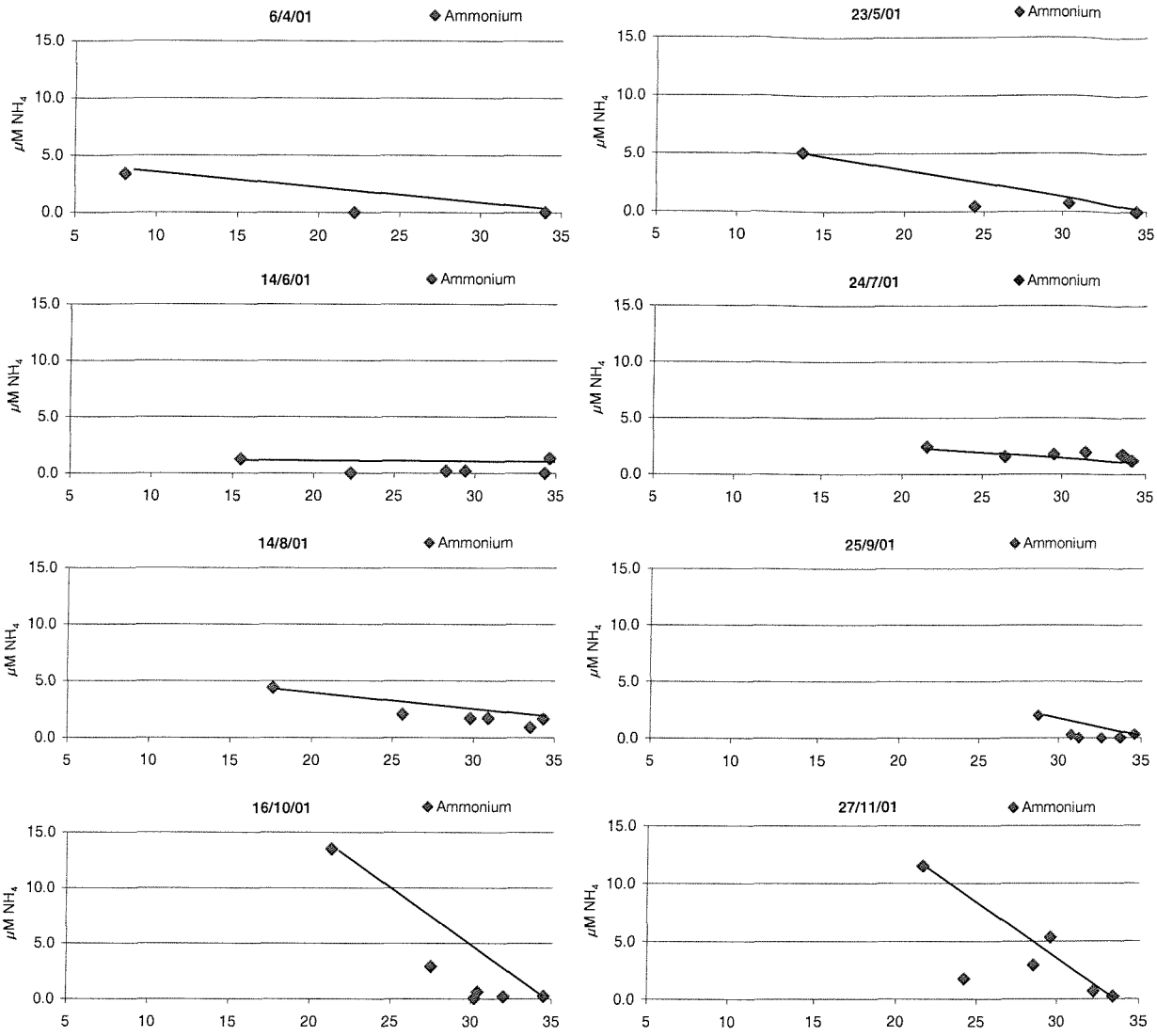






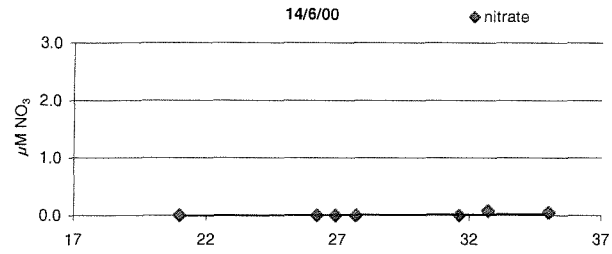
APPENDIX 7

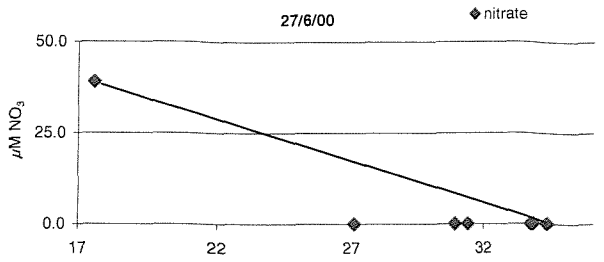
Ammonium (μM) versus salinity plots for each day of sampling throughout the Fleet lagoon in 2001
Lines connect data from station 1 and 6

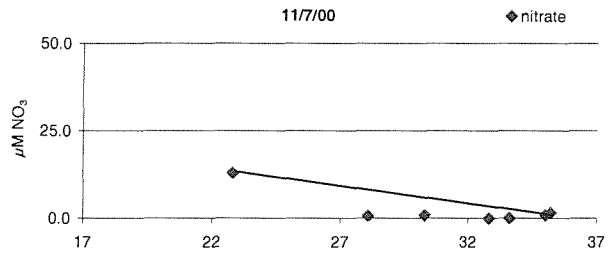


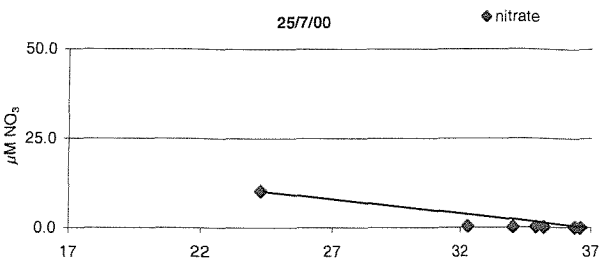
APPENDIX 8

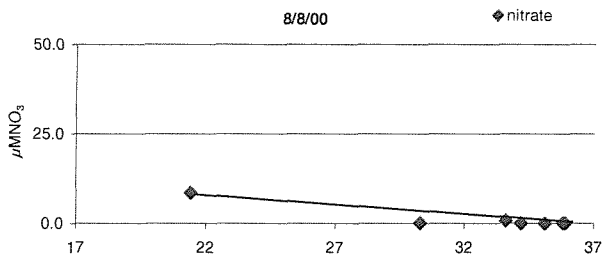
Nitrate+nitrite (μM) versus salinity plots for each day of sampling throughout the Fleet lagoon in 2000
Note change of scale. Lines connect data from station 1 and 7

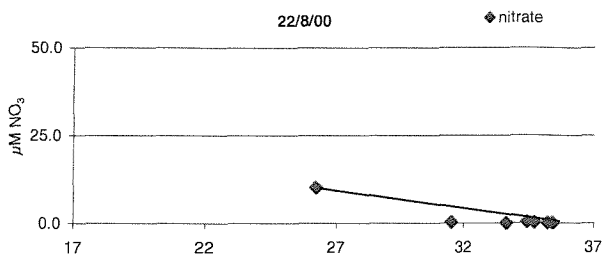


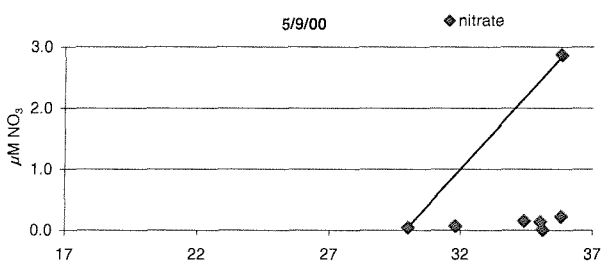


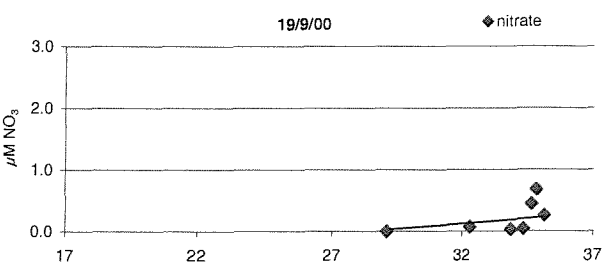


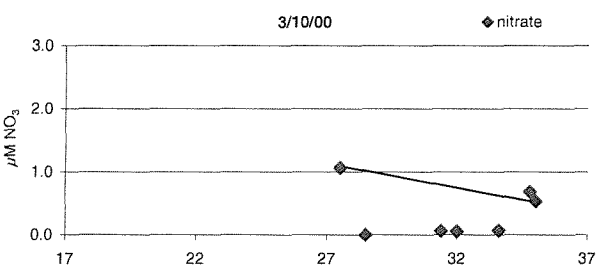


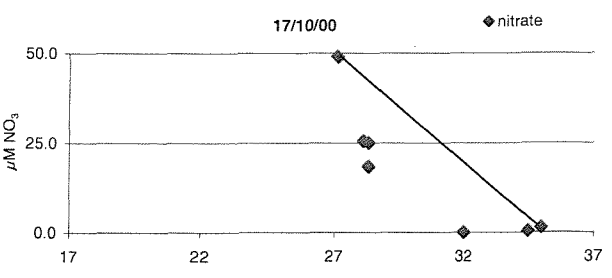


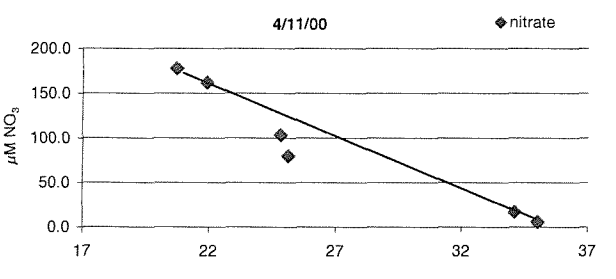






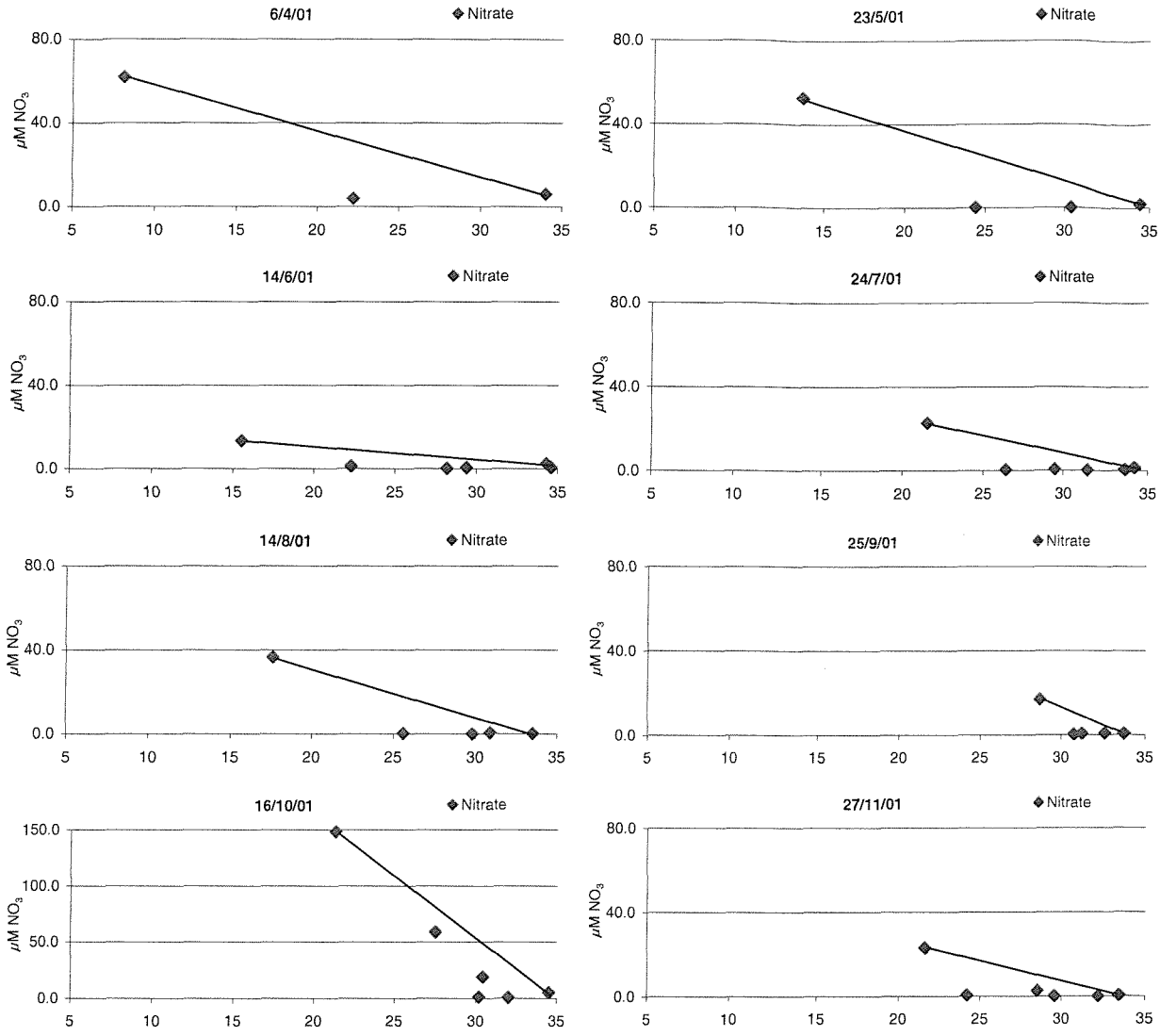






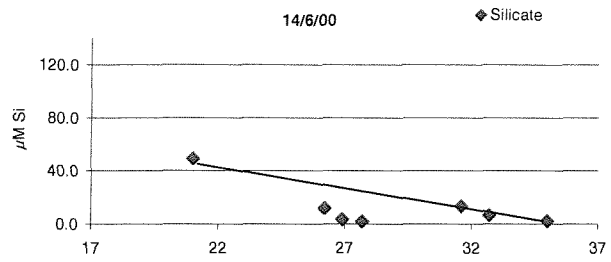
APPENDIX 9

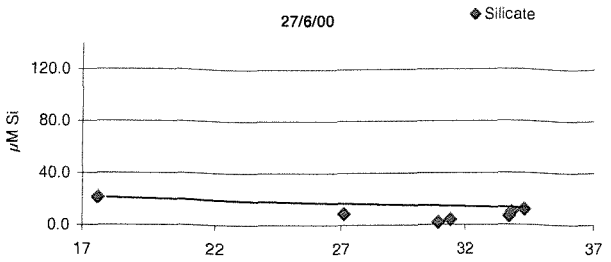
Nitrate+nitrite (μM) versus salinity plots for each day of sampling throughout the Fleet lagoon in 2001
Note change of scale. Lines connect data from station 1 and 6

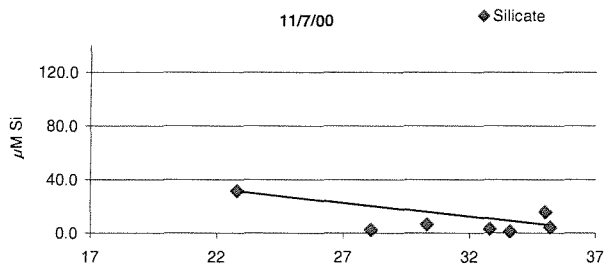


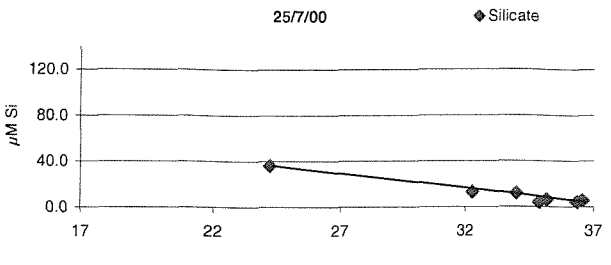
APPENDIX 10

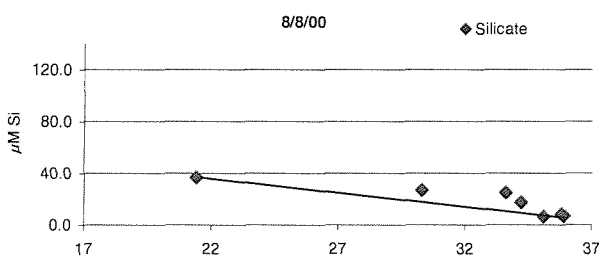
Silicate (μM) versus salinity plots for each day of sampling throughout the Fleet lagoon in 2000
Lines connect data from station 1 and 7

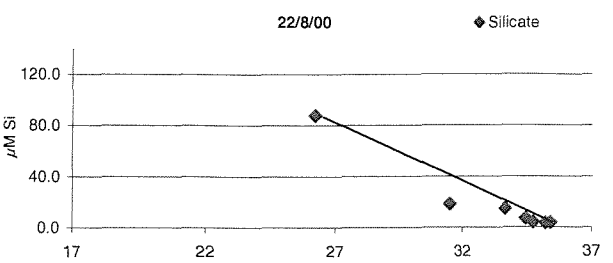


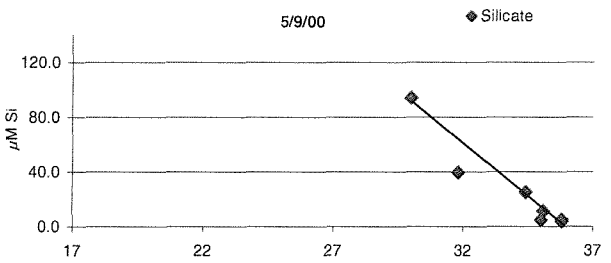


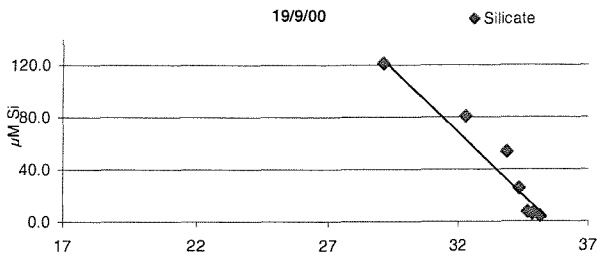


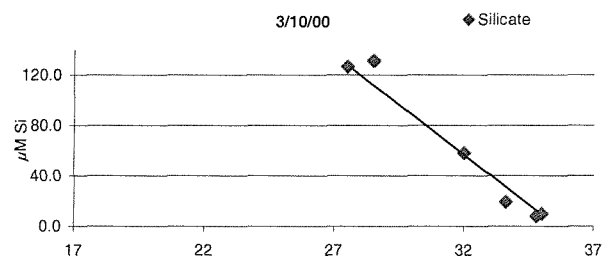


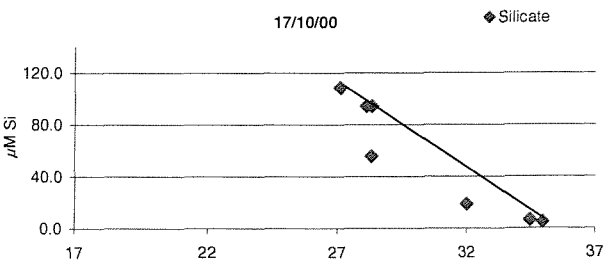


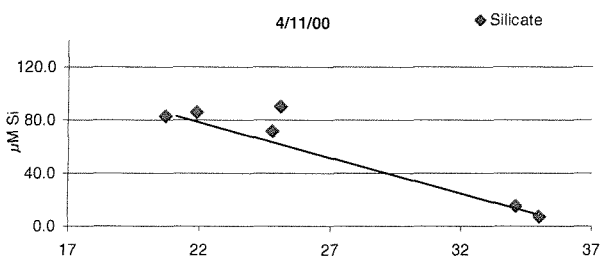






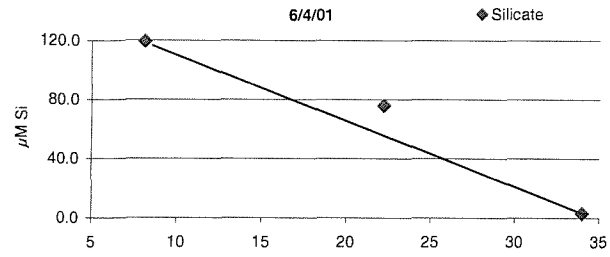


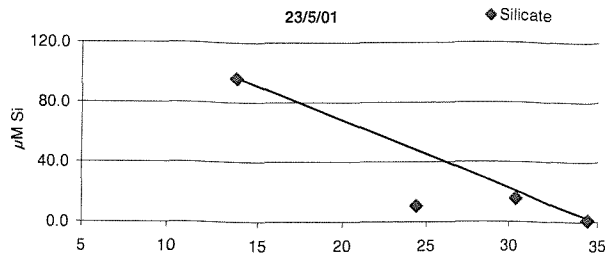


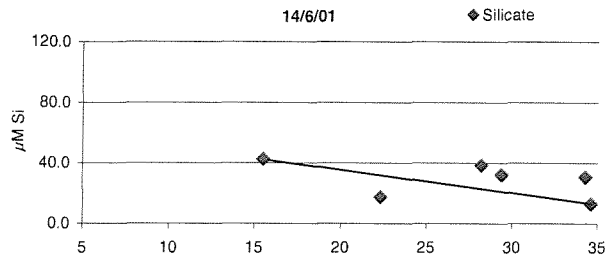


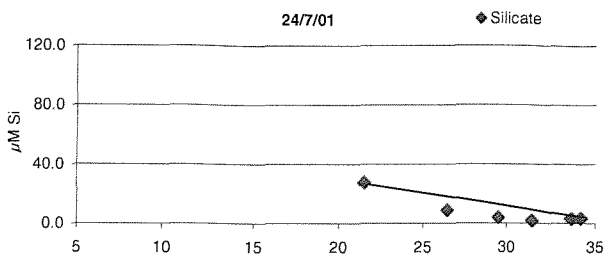
APPENDIX 11

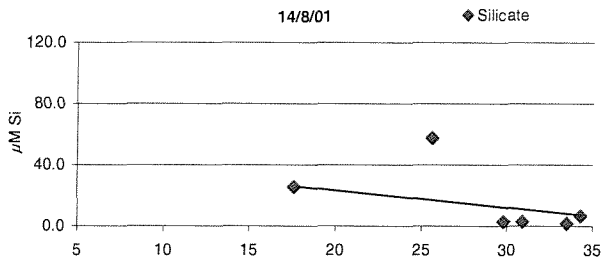
Silicate (μM) versus salinity plots for each day of sampling throughout the Fleet lagoon in 2001
Note change of scale. Lines connect data from station 1 and 6

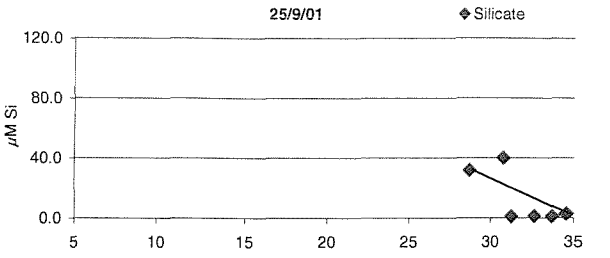


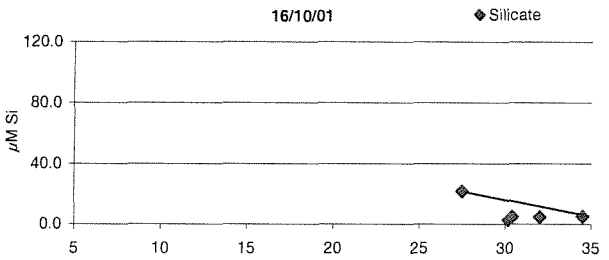


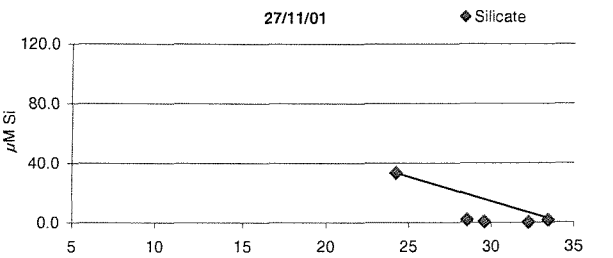








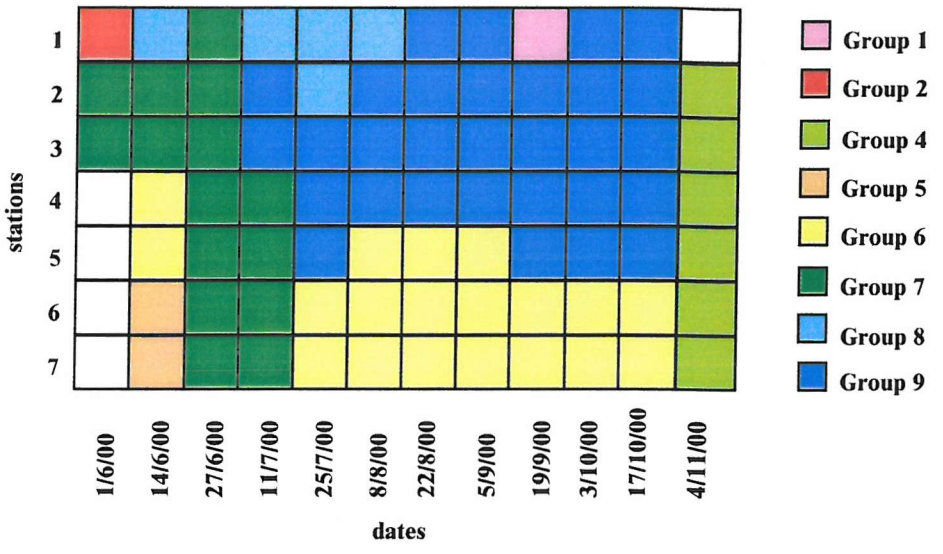




APPENDIX 12

Graphic representation of the distribution of groups from cluster3 (a) and cluster6 (b) with respect to sampling dates and station. In (a) group 3 of cluster3 was not represented as it refers to the only sample collected in 18/5/00 from station 3. Blank squares represent missing data. See Tables 3.4 and 3.5 for a list of the species contributing for each group.

a



b