Dynamics of harmful *Rhizosolenia* cf. *chunii* blooms in Port Phillip Bay

Final Report

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Project No. 96/264

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

MARINE & FRESHWATER RESOURCES INSTITUTE

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OBJECTIVES:

1. To document the frequency, intensity, duration and spatial occurrence of *R.* cf. *chunii* blooms in Port Phillip Bay from 1987 to 1995.

2. To investigate the environmental factors that influence the onset, development and timing of *R.* cf. *chunii* blooms in Port Phillip Bay.

3. To recommend growing/ harvesting and environmental management strategies to reduce the adverse impact of *R.* cf. *chunii* blooms on the marketability of cultured mussels from Port Phillip Bay.

NON-TECHNICAL SUMMARY:

Blooms of the diatom *R.* cf. *chunii* have occurred throughout much of Port Phillip Bay in 1987, 1993, 1994 and 1997, and probably also in 1975. When ingested by bivalve shellfish such as mussels and oysters, the alga imparts an intensely bitter taste to the shellfish rendering them unsuitable for human consumption. The bitterness is very persistent and some mussels have remained unmarketable for many months. High shellfish mortality may also occur. Such blooms threaten the viability of the mussel culture industry in Port Phillip Bay.

Cells of *R.* cf. *chunii* are always observed in June and July each year, but blooms have only occurred from early August to early October. Most major blooms developed first in the Geelong Arm, then spread in a clockwise (and possibly easterly) direction around the Bay, and took 2-4 weeks to reach sites in the north and east.

A large database of phytoplankton, water quality, meteorological, hydrological and effluent inflow data, from 1987 to 1995, was analysed to investigate the environmental factors that influence the onset and development of *R.* cf. *chunii* blooms in Port Phillip Bay. Despite variable environmental conditions in mid to late winter between years, the strong interannual synchronization of bloom events when they occurred suggested that specific seasonal events of photoperiod, water temperature, wind strength and nutrient

discharges acting in concert were necessary to provide the background conditions to trigger cyst germination and enhance bloom development. Day lengths of about 10 h and minimum annual water temperatures occur in late July/ early August. Wind strength during this period appeared sufficient to resuspend cysts into the water column, at least in the shallower Geelong Arm of the Bay, in all years with the possible exception of 1988. Nutrient discharges from the sewage treatment plant at Werribee increase significantly in winter and early spring, and were sufficient to enable *R.* cf. *chunii* to bloom in late winter. Furthermore, the proximity of the Western Treatment Plant is considered to be a major reason why blooms first appear in the Geelong Arm. However, blooms do not occur every year. A bloom was not observed in 1995, a year when the increased nutrient outflows in winter failed to reach the Geelong Arm.

While we did not find a single factor identifying bloom years from non-bloom years, two consistent indicators observed during bloom years were the decreased salinity within the Geelong Arm and Corio Bay and the similarity in the composition of the phytoplankton community 4 - 6 weeks prior to bloom initiation. *Rhizosolenia* cf. *chunii* blooms developed in four (1975, 1987, 1993 and 1994) of six years when water salinity at Clifton Springs was <34.5 in July and/or August; years 1995 and 1996 were the exceptions. Salinity was >34.5 in August-September of bloom year 1997, although salinity did fall below 33.75 eleven months prior to bloom initiation and similar low salinities were recorded around the late springs or summers immediately before the 1993 and 1994 blooms. A low relative abundance of diatoms among the phytoplankton community during June and July, combined with relatively high concentrations of the four taxa, *Plagioselmis prolonga*, *Teleaulax acuta*, *Pyramimonas* spp. and *Katodinium rotundatum*, were found to occur only during bloom years (useable data available from 1990 - 1997).

Consequently, an early warning system is proposed based on salinity: we predict that blooms will develop if salinity decreases to < 34.5 in July and /or August, or if salinity falls below 33.75 sometime during the eleven months before August. A low relative abundance of diatoms in the phytoplankton during June and July, combined with relatively high concentrations of the four taxa, *Plagioselmis prolonga*, *Teleaulax acuta*, *Pyramimonas* spp. and *Katodinium rotundatum*, may also indicate a greater likelihood of a bloom developing in August of the same year.

Research should be commissioned to: 1) investigate methods to rapidly purify mussels of the bitter-tasting compounds on a land-based facility. 2) isolate the unknown compound(s) responsible for the bitter taste in shellfish, and to develop an analytical method to measure the concentration of the bitter-tasting compound(s) or associated 'biomarkers' present in shellfish tissues as a determinant for marketability.

It is suggested that phytoplankton and salinity monitoring should be continued to provide detailed data on future bloom events and to improve predictive capacity.

BACKGROUND:

Mussel farming in Port Phillip Bay was first established in 1983. The Victorian growers quickly established a thriving industry, which was soon producing the bulk of the mussels grown in Australia. However, after several good years of production, in August 1987 a widespread and dense bloom of the diatom *R.* cf. *chunii* occurred in the Bay for about two months. Coincident with this bloom, bivalve shellfish such as mussels, flat oysters and scallops, throughout much of the Bay, developed an intensely bitter taste. The bitterness was very persistent in mussels and hence they became unmarketable for many months. At least 500 tonnes of cultured mussels, valued at over $1 million, had to be discarded. This represented almost the whole year's crop. All aquaculture zones in Port Phillip Bay were affected, but mainly those in the Geelong Arm in the west and Beaumaris in the northeast (Fig. 1b).

There were no reports of illness following consumption of bitter shellfish. The compounds responsible for the bitter taste appeared to be concentrated in the digestive gland of mussels, and this gland showed extensive inflammation and degeneration. Digestive gland lesions evident in mussels became progressively more severe as time passed, and high shellfish mortality was observed some 3-8 months after the algal bloom had disappeared. Scallops remained marketable throughout the bloom as scallop viscera is usually discarded and not eaten. However, split 'roe-on' scallops were very slightly bitter because of the small loop of intestine present in the roe. Parry *et al.* (1989) presented strong indirect evidence that the bitterness and shellfish mortality were caused by the *R.* cf. *chunii* bloom.

In early August of 1993 and 1994 further blooms of *R.* cf. *chunii* developed in the Bay and bitter-tasting shellfish was soon reported from areas where the algae appeared. These further outbreaks confirmed *R.* cf. *chunii* as the causative organism. The mussel culture industry had to again immediately cease harvesting. Only relatively minor tissue damage occurred on these two occasions, and the mussels remained in fairly healthy condition. However the bitter taste again remained in the mussels for many months after the end of the blooms in late September/ early October, and additional crop losses occurred, particularly in the Geelong Arm. Although many mussels were sold after the 1993 and 1994 blooms, there were customer complaints about inconsistent product quality and Port Phillip Bay mussels soon lost their previous high-quality image in the marketplace. Flat oysters, although then occurring in increasing numbers following a marked population decline due to bonamiasis, were also too bitter to market.

From a shellfish industry viewpoint, blooms of *R.* cf. *chunii* are arguably the most damaging of all algal blooms to occur in Port Phillip Bay. Other blooms have resulted in a temporary suspension of shellfish harvesting due to the production of potentially fatal PSP toxins, but these blooms do not cause high shellfish mortality or lengthy periods of contamination resulting in substantial crop losses. Furthermore, as *R.* cf. *chunii* has bloomed three times in the ten years between 1987 and 1996 (and probably also in 1975), it is obviously here to stay. The industry must therefore learn to live and adapt to the problem.

NEED:

From a shellfish industry viewpoint, blooms of *R.* cf. *chunii* are arguably the most damaging of all algal blooms to occur in Port Phillip Bay. Other blooms have resulted in a temporary suspension of shellfish harvesting due to the production of potentially fatal PSP toxins, but these blooms do not cause high shellfish mortality nor lengthy periods of contamination resulting in substantial crop losses. Furthermore, as *Rhizosolenia* has now bloomed three times in the past 10 years (and possibly also in 1975), it is obviously here to stay. The industry must therefore learn to live and adapt to the problem.

After the 1994 *R.* cf. *chunii* bloom a "Mussel Working Party" was established with industry and government representation and chaired by Mr. Gary Spry MP. The then Minister for Natural Resources, Mr. Geoff Coleman, was advised that the future economic viability of the mussel culture industry in Port Phillip Bay (particularly in the Geelong Arm) was in doubt. The Working Party recommended that the dynamics and causes of *R.* cf. *chunii* blooms in Port Phillip Bay should be investigated, and that a reliable early warning system should be implemented to advise mussel growers of the onset of future *R.* cf. *chunii* blooms. Pacific oyster culture by Cheetham Salt at Lara could also be affected by the blooms, since Geelong Arm water is used to fill the land-based culture ponds; a similar warning system is therefore necessary for this developing industry. Though scallop harvesting by dredge has been prohibited in Port Phillip Bay from 1998, the future of any other wild stock harvesting strategy will also be threatened by continuing blooms.

MAFRI has accumulated an extensive database of phytoplankton and environmental information which, when analysed with additional meteorological and hydrological data, may enable the causes of bloom development to be understood. Depending on the main causal factors, it may be possible to effect some control over future blooms, for example by altering the timing and/or point of discharge of sewage effluent from the Western Treatment Plant at Werribee. Alternatively, it may allow prediction of when blooms are likely to occur, leading to farming and harvesting strategies by which the Geelong Arm of Port Phillip Bay (in particular) can continue as a viable mussel growing area.

METHODS:

Analytical procedures

A substantial database was created for this investigation.

Extensive water quality data from seven sites within Port Phillip Bay (Fig. 1a), comprising nutrients, phytoplankton species identification, abundance and pigment concentrations and physical measurements was collected on a routine fortnightly basis between January 1990 and December 1995 and on a less frequent and comprehensive basis between September 1987 and December 1989. During the preparation of this report, later samples for phytoplankton identification and abundance were collected on a routine fortnightly basis from the maintained sites between January 1996 and December 1997 and the data obtained from this was added as an adjunct to this work. All these data were available at MAFRI. Nutrients were measured because of their critical role in the growth of plants, and physical parameters because they exert control over the timing or rate of growth. The inorganic nutrients ammonium, nitrite, nitrate, phosphate, and silicate are those most readily available for phytoplankton growth. However, organically-bound nitrogen and phosphorus may also be available to certain types of plankton (Fisher and Cowdell 1982), while particulate nitrogen, phosphorus and carbon can give information on the proportion of particulate matter in the water column made up of living cells. Phytoplankton pigments, as estimates of plant biomass, included chlorophyll *a*, chlorophyll *b*, chlorophyll *c*, carotene, living chlorophyll *a* and phaeophytin (degradation products resulting from the digestion of chlorophylls by zooplankton). Physical parameters included salinity, water temperature, water column light attenuation, suspended solids as non-filterable residue and secchi depth.

Salinity, temperature and depth were measured with a Yeo-Kal SDL submersible data logger, run in direct reading mode. Data were fed to a computer for display and storage. Water samples were collected from specific depths by Niskin bottle for calibration of salinity. In the laboratory, a Yeo-Kal model 601 mark IV bench salinometer measured salinity to a precision of 0.003. Temperature was calibrated to 0.01°C against a certified Sensoren Instrument Systems digital reversing-thermometer.

Chlorophyll fluorescence was measured with a Seatech submersible fluorometer or with a Turner Designs model 10 fluorometer fitted with a flow-through cell. Data were digitised with a Data Electronics DT 200 data logger and stored on computer. Fluorescence was converted to chlorophyll *a* by least-squares linear regression based on calibration against chlorophyll analyses of water samples collected at the out-flow of the fluorometer cell at noted times. Chlorophyll samples, collected on Whatman GF/C glass fibre filters by gravity filtration, were initially frozen for storage. The chlorophyll was then extracted by ultrasonification in ice-cold 90% acetone, and the concentration determined by polychromatic spectrophotometry (Strickland and Parsons 1972) using the equations of Jeffrey and Humphrey (1975).

Depth integrated water samples, considered as representative of the whole water column, were collected by submersible pump for nutrient analyses. Inorganic nutrients were measured by segmented-flow colorimetry on unfiltered water. Ammonium (NH_4) was

measured by the phenol-hypochlorite method of Solorzano (1969) as automated by Technicon (1973a). This method has been significantly modified by MAFRI to eliminate salinity-dependent sensitivity. Nitrite (NO_2) was measured by the method of Bendschneider and Robinson (1952) as automated by Technicon (1972). Nitrate (NO_3) was also measured as NO_2 after reduction with a cadmium coil (Morris and Riley 1963), again as automated by Technicon (1972). Phosphate (PO_4) concentration was determined by the molybdenum blue method of Murphy and Riley (1962) as automated by Technicon (1973). Monomeric reactive silicate (SiO_4) was measured by the method of Koroleff (1972) as automated by Technicon (1973b). Standards and system blanks were analysed frequently to detect drift and changes in sensitivity.

Total phosphorus (TotP) and Kjeldahl nitrogen (KjlN) were determined by H_2SO_4/H_2O_2 digestion (Nicholls 1975). Particulate nitrogen (PrtN) and particulate phosphorus (PrtP) were determined by the same method on samples collected on distilled, deionised water-washed glass fibre filters. Total organic carbon (TOC) was measured by a low-temperature UV/NDIR method on an Astra 1000 analyser, while particulate organic carbon (POC) was measured by the high-temperature combustion/NDIR detection of samples on combusted glass fibre filters.

Photosynthetically-active radiation (PAR) attenuation is an electronic measure of the amount by which light suitable for plant growth is attenuated as it travels through the water column and was measured using a Licor LI 192SB submersible sensor connected to a Licor LI 1000 logger. An integration time of three seconds was used to smooth the output, while a deck-mounted sensor accounted for variations in incident radiation. Readings were collected from just above and below the water surface, and at 0.5, 1, 2, 5, 10, 15 and 20 m, and an attenuation coefficient was calculated from the slope of PAR against log of depth.

Suspended solids, or non-filterable residue is a measure by gravimetric analysis on glass fibre filters (Anon. 1979) of the concentration of particles in the water column which may reduce water clarity. Secchi depth also provides an indication of light penetration and is a measure of the depth to which a white disc can be lowered into the water column before it disappears from view.

Oxidised nitrogen was calculated as [NO_2+NO_3]; dissolved inorganic nitrogen (DIN) as [NH_4 +NO_2+NO_3]; dissolved organic nitrogen (DON) as [KjlN-DIN-PrtN]; dissolved organic phosphorus (DOP) as [TotP-PO_4-PrtP]; and dissolved organic carbon (DOC) as [TOC-POC].

In Port Phillip Bay, NH_4, NO_2, NO_3 and SiO_4 are "dissolved" (filtration through a Whatman GF/C filter with nominal pore size of 1 µm does not reduce their concentrations), while up to 20% of PO_4 detected by the colorimetric method is associated with particles greater than 1 *u*m in size (A. Longmore, unpublished data).

Experiments at MAFRI have indicated that freezing of Port Phillip Bay water is normally an adequate preservation method for both short-term (< 24 hours) and longer-term (3-9 months) storage of unfiltered samples for subsequent analysis of NH_4, NO_2, NO_3, PO_4 and SiO_4 concentration (A. Longmore, unpublished data).

Information on *R.* cf. *chunii* was extracted from a large phytoplankton database, which contained routine cell concentration data for 205 species or taxa (Magro *et al.* 1996). A one litre sample of surface water was collected at each site on each sampling occasion, kept at ~ 15°C and transferred within 24 hours of collection to the School of Botany, University of Melbourne, where phytoplankton identifications and cell counts were performed by or under the supervision of Dr. David Hill. The one litre water samples were concentrated by continuous centrifugation to a final volume of 7 mL. Cell counts were made using a haemocytometer with a cavity volume of 0.9 mm^3 (3 mm x 3 mm x 0.1 mm). At least 200 cells were counted in each sub-sample, and the numbers were extrapolated to estimate the concentration of each species in the original one litre water sample. Species identifications were made with the aid of a light microscope, although some identifications were periodically verified using an electron microscope.

Meteorological data included rainfall, wind speed and direction, solar radiation, total cloud cover, and air temperature (wet bulb, dry bulb and dew point). Those parameters measured hourly were reduced to a daily average. Rainfall, measured daily at 0900 h, was obtained for several stations around Port Phillip Bay. As high concentrations of *R.* cf. *chunii* were first observed at Clifton Springs (site 13 in the Geelong Arm) during 1993 and 1994, mean daily rainfall was calculated from data obtained from nearby rainfall stations at Portarlington, Geelong and Werribee. Daily wind speed and direction at Pt. Cook, situated between Werribee (site 11) and Williamstown (site 9), were estimated from hourly data. Daily solar radiation, and daily mean cloud cover and air temperature estimated from hourly readings taken between 0500 and 1700 h at the Laverton weather station (northeast of Werribee), were also included.

Weekly flow and nutrient load data from the Western Treatment Plant (WTP) were summed over 14-day periods.

Data analysis

Phytoplankton, hydrochemical, meteorological, WTP and river discharge data were combined to form one database. The period covered by the different parameters varied, but data for all variables were available from February 1990 to December 1995. Occasionally, one or more days separated the usually coincidental collection of water quality samples and phytoplankton samples, but such discrepancies have been ignored in the preparation of a database for the following analyses.

The data were first analysed graphically to identify patterns over time in *R.* cf. *chunii* cell concentration and several hydrochemical variables at Clifton Springs (site 13 in the Geelong Arm), and in meteorological and WTP discharge data. Analyses of variance were conducted using the General Linear Model (GLM) procedure, on SAS software (SAS Institute Inc. 1990), to identify significant effects. Phytoplankton data from Clifton Springs were also subjected to pattern analysis (Belbin 1990) to assess if the pattern of assemblages reflected bloom and non-bloom years. A double square root transformation was applied to the species abundance data for each site before analysis as a means of reducing the imbalance of large numerical differences between the species populations.

RESULTS:

1. Distribution, intensity and duration of *Rhizosolenia* cf. *chunii* blooms

Annual occurrence

Cells of *R.* cf. *chunii* were found in Port Phillip Bay in 1987, 1989, 1991, 1992, 1993, 1994 and 1995 (i.e. all years except 1988 and 1990). There is no prescribed cell concentration which is commonly used to indicate 'bloom' conditions, but in this study we have defined a bloom as an event a with a minimum *R.* cf. *chunii* cell concentration of 50,000 cells L^{-1}. Based on this definition the years 1987, 1993 and 1994 are defined as bloom years (Table 1) and are coincident with the years when bitter taste was detected in mussels.

Bloom intensity

Cell concentrations of *R.* cf. *chunii* from the 7 routinely sampled sites were averaged by month for those years in which it was observed (Fig. 2). As phytoplankton sampling only started in September 1987, in response to reports of bitter-tasting shellfish, means for months in 1987 before September are not available.

It is possible for a species to have a relatively high mean cell concentration but still not dominate the phytoplankton. The concentration of *R.* cf. *chunii* cells expressed as a percentage of the total phytoplankton cell concentration during the month and year it was observed is shown in Table 2. In the bloom years 1987 and 1994, *R.* cf. *chunii* cells accounted for a substantial proportion of the total cell concentration. The greatest cell concentrations of *R.* cf. *chunii* occurred during August, September and October, although a small number of cells were observed in December 1992.

Duration and spatial occurrence

Phytoplankton sampling in 1987 started in early September as a response to industry concerns regarding the bitter taste of mussels, and there was consequently no sampling before and during the early stages of the large bloom event of that year. High cell concentrations were found in September 1987, but by early October the bloom had disappeared. In both 1993 and 1994 the blooms of *R.* cf. *chunii* first appeared at Clifton Springs in the Geelong Arm of Port Phillip Bay, later spreading in a clockwise direction around the Bay (Fig. 3). Cell concentrations at Wooley Reef and Dromana, sites on the eastern side of the Bay, were low during 1993 and especially low in 1994.

2. Inputs from Western Treatment Plant

The effluent flow and nitrogen (N), phosphorus (P) and organic carbon loads from the WTP each fortnight (based on sum of weekly values) from 1990 to 1995 are shown in Figs. 4a and 4b. Also included is the fortnightly DIN:DIP ratio, which may be used to

infer whether the WTP discharge is nutritionally balanced for diatom growth. Redfield *et al.* (1963) suggested that diatoms grow at optimum rates when exposed to a nutrient supply in the ratio 16N:1P:17Si.

There is a similarity in the annual patterns of effluent flow and nutrient loads from the WTP (Figs. 4a-b) and is most likely indicative of the stable population of Melbourne. Ammonium is the nutrient with the most distinct seasonal pattern. Loads of this nutrient began to increase in April each year, four months before the occurrence of *R.* cf. *chunii* blooms, and peaked in winter or early spring.

The observed variation in the DIN:DIP ratio indicates that the WTP effluent is always poor in N relative to P compared with the optimal ratio (16:1) required for diatom nutrition (Redfield *et al.* 1963). Diatom growth off the WTP is therefore more likely to be N-limited rather than phosphorus-limited. Nitrogen limitation is greatest in the February-April period each year.

Statistical tests were used to verify the above observations. An ANOVA for unbalanced data was conducted on monthly mean data from January 1990 to December 1995 to investigate monthly and annual differences of WTP variables. The dependent variables were effluent discharge (Flow), and loads of NH_4, NO_2, NO_3, PO_4, organic nitrogen (OrgN), TotP and TOC. Bartlett's tests for homogeneity of variances indicated that NO_2, NO_3, PO_4, OrgN, TotP and TOC required a log transformation before analysis. The model used on monthly data from January 1990-December 1995 was:

$$\text{Flow } NH_4\ NO_2\ NO_3\ PO_4 \text{ OrgN TotP TOC} = \text{Year} + \text{Month} + \text{Year*Month} + \text{error}$$

The levels of significance for the various parameters from the WTP on the independent variables year, month and year-month interaction are shown in Table 3, while the order of results of annual mean and monthly mean values, and temporal groupings derived from Duncan's tests, are shown in Tables 4 and 5 respectively. The Duncan's test is a multiple range test which orders the means of the dependent variables (such as flow) in hierarchical order of the independent variables (such as years, where in Table 4 the greatest annual mean flow was found to occur in 1990 and the lowest annual mean flow occurred in 1994) and tests whether there was a significant difference among the population means (shown in Table 4 where 1990 had greater mean flow than 1992, but as indicated by the letter 'A' under both 1990 and 1992, both years were not significantly different from each other).

Table 3 shows there to be significant differences ($p<0.05$) between the annual mean values of all parameters except for TotP and for there to be significant differences between the monthly mean values of all parameters except for OrgN.

The results from the Duncan's test of annual and monthly parameters, shown in Tables 4 and 5 respectively, can indicate where the significant differences among the annual and monthly means lie. The numerical differences as shown in the hierarchical order of the annual means (Table 4) indicate a trend of the two bloom years of 1993 and 1994 having adjoining values for the parameters of NH_4, NO_3, PO_4, OrgN and TotP. However, only with the NO_3 load are 1993 and 1994 as an adjoining pair significantly differently from other years (shown by the 'B' value under 1993 and 1994 solely). For those parameters

where 1993 and 1994 were not adjoining, such as for flow, NO_2 and TOC, neither of those bloom years were significantly different to a number of other years (Table 4).

Duncan's hierarchical ordering of monthly mean values of the parameters associated with WTP (Table 5) show that the highest mean monthly flows and higher mean monthly levels of NH_4, NO_3, PO_4, TotP and TOC occurred within June and July, the two winter months preceding August which appears to be the month of bloom initialisation. However, June and July were not significantly different from other months within each parameter tested.

3. Physico-chemical parameters in Port Phillip Bay

Data in this section are provided only for Clifton Springs (site 13), where high concentrations of *R.* cf. *chunii* were first observed during bloom years. The physical and chemical parameters included are those which may enhance (e.g. nutrients) or reduce (e.g. light attenuation or suspended solids) algal growth. The nutrient data are water-column concentrations, which are potentially available for phytoplankton utilisation.

Plots of the fortnightly concentrations of NH_4, NO_2, NO_3, SiO_4 and PO_4 from 1990 to 1995 are shown in Figure 5a. Concentrations of all three forms of dissolved inorganic nitrogen peaked in early or mid-winter (June-July) in four of the five years, although an additional NO_3 peak occurred in August 1992. No peaks were observed in 1995. Less variation was apparent in PO_4 concentration both throughout the year and between years. Concentrations of SiO_4 were usually high in autumn, but declined through winter and were near zero in August or September of most years.

As diatoms require N:Si:P in the ratio 16:17:1 for optimal growth (Redfield *et al.* 1963), the temporal variation in the inorganic nutrient ratios at Clifton Springs (Fig. 5b) can indicate the likelihood of nutrient limitation of phytoplankton growth. The N:P ratio was uniformly low in all years; although small peaks were observed in winter, the N:P ratio was never higher than 5:1 (optimum 16:1). This indicated that N limitation was more likely to have occurred than P limitation. Similarly, the Si:P ratio was always much lower than the optimum of 17:1, indicating that Si limitation was always more likely to have taken place than P limitation. However, the N:Si ratio is more informative than either the N:P or Si:P ratios because Port Phillip Bay is P-rich and the chance of P limitation occurring is remote. A N:Si ratio <1 indicates that N limitation is possible, while a ratio >1 indicates that Si limitation was more likely to occur. Except for 1992, there does appear to be Si limitation occurring during the winter periods for the years 1990-1995 (Fig. 5b) with N:Si ratios between 2:1-7:1. This is in contrast with the remaining period of each year where N limitation is indicated with N:Si ratios usually between 0.2:1-0.4:1. In the absence of other controlling factors, Si limitation would favour the development in winter of phytoplankton species which do not require silicate for growth (e.g. dinoflagellates), and may explain why the *R.* cf. *chunii* blooms in 1993 and 1994 collapsed in September. However, N limitation was more likely than Si limitation in early winter before the *R.* cf. *chunii* blooms developed.

As Port Phillip Bay phytoplankton may also utilise organic nutrients for growth (Fisher

and Cowdell 1982), plots of fortnightly concentrations representative of the whole water column, at Clifton Springs, are shown in Figure 5c. Organic N concentrations were relatively high in winter, and small peaks were evident in early to mid winter of most years. Concentrations of PrtN were the most variable of the 'organic' parameters, several large peaks occurring, and concentrations were observed to increase between early to mid winter. Particulate P was also somewhat variable, several peaks being evident in winter and spring, while TotP displayed little variation (as did TOC) although concentrations were relatively high in mid to late winter.

An ANOVA was conducted on monthly mean data, after transformation of the entries for nine of the eleven chemical parameters (NH_4, NO_2, NO_3, OrgN, TotP, TOC, PrtP, PrtN, and POC) was required to satisfy homogeneity of variance assumption of ANOVA. The model used on monthly data from January 1990-December 1995 was:

NH_4 NO_2 NO_3 PO_4 SIO_4 OrgN TotP TOC PrtP PrtN POC
= Year + Month + Year*Month + error

The levels of significance of the dependent chemical parameters on the independent variables year, month and year*month interaction are shown in Table 6, while the order of results of annual mean and monthly mean values, and comparison of means showing any significant differences between years and months from Duncan's tests, are shown in Tables 7 and 8 respectively.

Significant differences (<0.05 level) occurred between the annual mean values for all parameters and between the monthly mean values for all parameters except for TOC, PrtP and POC (Table 6). The year*month interaction was also significant (<0.05) for all parameters, again with the exception of TOC, PrtP and POC.

Duncan's testing of annual means showed that only for SiO_4 did the bloom years of 1993 and 1994 adjoin but even so, were not significantly different from other years when tested with this parameter (Table 7). Mean concentrations of NH_4, OrgN and TOC were highest in 1993, and significantly different to lower 1994 values. Organic N was the only parameter for which the 1993 mean was significantly higher than that of all other years. Lowest mean concentrations of PO_4, TotP, PrtP, PrtN and POC occurred in 1994, however with each parameter tested, it was found that 1994 was not significantly different to two or three other years.

Duncan's testing of monthly mean values (Table 8) were in general agreement with observations based on the graphical presentations (Figs. 5a and 5c). Monthly mean concentrations for seven of the eleven chemical parameters (NH_4, NO_2, NO_3, PO_4, OrgN, TotP and PrtN) were highest in July. Concentrations of the three forms of dissolved inorganic nitrogen (NH_4, NO_2, NO_3) were significantly higher in July and June compared to other months.

The temporal distribution of SiO_4 was different from that for the other inorganic dissolved nutrients NH_4, NO_2, NO_3 and PO_4 (Table 8). Mean values of SiO_4 were greater in all the months preceding June and July, but not significantly. However, August and September formed a group with lowest mean concentrations and significantly different from June and

July values. These results confirm the earlier observation that SiO_4 concentrations decreased fairly rapidly between July and August/ September.

Fortnightly measurements of physical factors that may effect phytoplankton growth, i.e. salinity, temperature, PAR attenuation, secchi depth and non-filterable residue (NFR), are shown in Fig. 5d.

Rainfalls in late 1992 and in 1993 resulted in lower water salinity throughout 1993 and in the first half of 1994. The salinity in June 1993 and June 1994 was about one unit lower than in other years. Neither PAR attenuation nor secchi depth indicated variation in light climate between bloom and non-bloom years or before *R.* cf. *chunii* blooms. Except for a large peak in July 1990, there was also little variation in NFR, for the period March-July, between bloom and non-bloom years. Temperature was highest at the start of 1993, but there appeared little difference between years after March.

An ANOVA was conducted on monthly mean data for the physical parameters NFR, Secchi depth, PAR attenuation, temperature (Temp) and salinity (Salin), with NFR data log transformed before analysis to satisfy homogeneity of variance assumption of ANOVA. The model used for the physical parameters was:

NFR Secchi PAR Temp Salin = year + month + year*month + error

The levels of significance of the physical parameters on the independent variables year, month and year*month interaction are shown in Table 9, while the order of comparison of means from Duncan's tests, are shown in Tables 10 and 11.

Significant differences ($p < 0.05$) were found between the annual mean values of NFR, PAR, Temp, and Salin, and between the monthly mean values of Temp and Salin (Table 9). The year*month interaction for PAR and Salin were also significant.

Duncan's testing of the annual means of the physical parameters showed that only salinity differentiated the *R.* cf. *chunii* bloom years of 1993 and 1994 from the rest (Table 10). The lowest annual mean salinity occurred in 1993 (33.8 parts per thousand), followed by 1994 (34.1 parts per thousand), and both years were significantly different from each other and from all other years. The concentration of particles (measured as NFR) in the water column was moderately low, but not significantly, in the two bloom years, but was lowest in 1992. Water clarity (as measured by PAR attenuation) was moderate to high in the bloom years, but the clarity was significantly higher in 1990; low light attenuation results in high water clarity. No significant differences were found in Secchi depths. Annual mean water temperature was significantly higher in 1993 compared with all other years.

Of the five physical parameters, only temperature could be used to differentiate the pre-bloom and bloom months (June, July, August and September respectively) from the rest (Table 11). Monthly mean temperatures were significantly lowest in July and August, while mean values in June and September were also significantly lower than the remaining 8 months.

4. Phytoplankton pigments

Chlorophylls *a*, *b* and *c*, and carotenoids are photosynthetic pigments, which enable plants to trap and utilise light. Chlorophyll *a* is the main photosynthetic pigment present in all phytoplankton taxa. Chlorophyll *b* is an important constituent of green algae, and a minor constituent in other taxa, while chlorophyll *c* is commonly found in brown algae and, in trace concentrations, in dinoflagellates. Carotenoids are accessory pigments, which enhance a plant's ability to utilise light at a greater range of wavelengths. Phaeopigments, on the other hand, are degradation products resulting from the digestion of chlorophylls by zooplankton, and at times of intense zooplankton grazing they may form a large proportion of the total plant pigment concentration.

Pigment concentrations can be used to estimate phytoplankton biomass and to indicate the relative importance of different taxon groups within the phytoplankton. Pigment ratios may also be used to assess the health of a phytoplankton community. A carotenoid:chlorophyll *a* ratio > 2.4 indicates an extreme N deficiency in the water column, while a ratio < 1.4 indicates that excess N is available for plant growth (Heath *et al.* 1990, Longmore *et al.* 1996).

Pigment concentrations at Clifton Springs from 1990-95 are shown in Fig. 6. Chlorophyll *a* concentrations were highest in winter-spring each year. The peaks in early August in 1993 and 1994 resulted from the *R.* cf. *chunii* blooms; other peaks occurred in the winter of 1991 (July) and 1995 (June), and in October 1990 and December 1992. In comparison to chlorophyll *a*, chlorophylls *b* and *c* were always minor constituents. The distribution pattern for chlorophyll *c* and carotenoids was similar to that for chlorophyll *a*, while both phaeophytin and living chlorophyll *a* concentrations indicated that more than 90% of the photosynthetic material was associated with living (i.e. ungrazed) cells. Living chlorophyll *a* is estimated by subtracting the phaeophytin concentration from the total chlorophyll *a* concentration. The carotenoid:chlorophyll *a* ratio indicates that cells were usually growing under slight N stress, although at times N was not limiting. There was nothing in the pigment distributions to show that the years 1993 and 1994 were different to other years.

An ANOVA was conducted on monthly mean data for chlorophyll *a* (ChlA), chlorophyll *b* (ChlB), chlorophyll *c* (ChlC), carotenoid (Carot), phaeophytin (Phaeo) and living chlorophyll *a* (LivA). Chlorophyll *b*, chlorophyll *c* and phaeopigment concentrations were log transformed before analysis to satisfy homogeneity of variance assumption of ANOVA. The model used on monthly averages of pigment concentrations was:

ChlA ChlB ChlC Carot Phaeo LivA = Year + Month + Year*Month + error

Results of ANOVA of dependent pigment parameters on the independent variables year, month and year*month interaction are shown in Table 12, while the order and comparison of annual and monthly mean values from Duncan's tests are shown in Tables 13 and 14 respectively.

Significant differences occurred between the annual mean concentrations for all

parameters except LivA, and between the monthly mean values for all parameters except Carot and Phaeo (Table 12). The year*month interactions were significant for all parameters.

Duncan's testing indicates that annual mean concentrations of all pigments (except for ChlC) were relatively high during 1993 compared to most other years although not significantly so. While 1994 had the lowest annual mean concentrations of all pigments (except for Phaeo), the values were not significantly different to isolate 1994 from other years. (Table 13). There was significant differences between 1993 and 1994 annual mean values of ChlB and Phaeo. Annual mean concentrations of ChlC were lowest in 1993 and 1994, although not significantly different from 1991.

The highest or second highest monthly mean concentrations of all pigments were in July (Table 14), with ChlA (the major phytoplanktonic pigment) concentrations in July being significantly greater than in other months.

5. Meteorological parameters

The total sum of regional rainfall in the summer period (December, January and February) preceding the bloom years of 1987, 1993 and 1994 was greater (246 mm, 256 mm and 272 mm respectively) than for the summer periods of other years (Fig. 7). Otherwise, there were no consistent features to particularly distinguish rainfall patterns in the bloom years from the non-bloom years. Heavy rainfall occurred during January and/or February for 1987, 1988, 1990, 1993 and 1994, but march and April were relatively dry. May was dry in 1993 and 1994, but extremely wet in 1987 (92.3 mm recorded over seven days). June was a fairly wet month in both 1987 and 1993, although falls in 1994 were light. Heavy rain fell in early July 1993 and late July 1987, but July 1994 was dry. Heavy rain also fell in mid-July 1990, although no *R.* cf. *chunii* bloom developed in that year.

Daily solar radiation and daily mean scalar wind speed for the month of July are shown in Fig. 8. In 1987 and 1993 there were periods of higher than average daily solar radiation, lasting for 1-2 days, alternating with periods of below-average radiation. In both years (and in 1991) radiation fell sharply at the end of July. These decreases coincided with periods of increased wind speed, indicating storm activity. However, only in 1987 did heavy rainfall occur in late July.

The variation in the meteorological parameters scalar wind speed (SWS), total daily radiation (TDR), total cloud amount (TCA), dry bulb temperature (DBT), wet bulb temperature (WBT), dew point temperature (DPT) and rainfall were investigated by ANOVA for the years 1985-1995. Rainfall data was log transformed prior to analysis to satisfy homogeneity of variance assumption of ANOVA. Weekly mean data were calculated for the six weeks before 4 August each year, as high concentrations of *R.* cf. *chunii* were first observed at Clifton Springs on 4 August 1993 and 3 August 1994. The model statement was:

SWS TDR TCA DBT WBT DPT Rain = Year + Week + Year*Week + error

The levels of significance ($p < 0.05$) of the dependent meteorological parameters on the independent variables year, week and year*week interaction are given in Table 15, while the order and comparison of weekly mean values for the six weeks before 4 August from Duncan's tests are shown in Table 16.

Significant differences occurred between the annual mean values for TDR, DBT, WBT and DPT in the six weeks before 4 August, and between the weekly mean values for the same parameters during the same six week period (Table 15). There were also significant year*week interactions for all parameters except TDR.

Duncan's testing showed no significance difference was found in SWS between any of the six weekly-periods before 4 August (Table 16). In addition, the raw data showed that the wind conditions in late July and August during bloom years were quite different. In 1987 strong winds (>7.5 m s^{-1}) occurred on 21 and 29 July, followed by relatively calm conditions from 2 to 19 August when wind strength was consistently under 5.0 m s^{-1}. In contrast, in 1993 there were seven days between 23 July and 21 August when wind strength was greater than 7.5 m s^{-1}, but no days during this period when wind strength was less than 5.0 m s^{-1}. Conditions in 1994 were different again. A very strong wind (>10.0 m s^{-1}) was recorded on 29 July followed by calmer weather, one further windy day on 12 August, and then a long period of calm with no further windy days until late September.

The entire 6-week period before 4 August falls after the winter solstice and hence the photoperiod (day length) would be increasing continuously during this time. This is reflected in the order of the weekly mean data for TDR (Table 16) with week 1 being the week immediately preceding 4 August. Weekly mean values were highest in weeks 1 and 2, although only week 1 was significantly different to week 3. There was no significant difference in TCA between any weekly means.

Mean WBT and DPT were significantly lower in week 2 compared with mean values for all other weeks (Table 16). The mean DBT was also lowest in week 2, but was not significantly different to that for two other weeks.

6. Phytoplankton species composition

A pattern analysis of phytoplankton species abundance data was conducted, using the software package PATN (Belbin 1990), to determine whether community compositions were similar between bloom years and less similar from non-bloom years.

Cell concentrations of all phytoplankton species observed at Clifton Springs were averaged in three 3-monthly periods (November-January, February-April and May-July) before 1 August for the years 1988-1995. The period before 1 August was of most interest because of the early August initiation of *R.* cf. *chunii* blooms in the Geelong Arm of Port Phillip Bay in 1993 and 1994. A 3-monthly time scale was selected as environmental conditions occurring in the preceding 100 days are of most significance in determining algal species succession (Harris 1986). Mean species concentrations in the 6-week period before 1 August of each year were also calculated and compared.

Dendrograms resulting from the pattern analysis for each of the four time periods are shown in Figure 9. The most similar community composition occurred between the six-week period before 1 August of 1993 and 1994. Similarity was also evident for the longer May-July period of 1993 and 1994, but populations were much less similar at other corresponding times in 1993-94. Community structures were less similar in all time periods between other years; two exceptions were the November-January period of 1991 and 1993, and the February-April period of 1989 and 1990.

In June and July of 1993 and 1994 diatoms (codes 2000-2999) comprised a very small percentage of the total phytoplankton cell concentration (Figs. 10 and 11). Diatom concentrations were also relatively low during these two months in 1992, although substantial diatom populations (other than *R.* cf. *chunii*) occurred in June and/or July in the other five years. Cryptophytes (codes 4000-4999) and prasinophytes (codes 7000-7999) were the dominant classes present in June and July of 1993, while cryptophytes alone dominated in June and July 1994 (Fig. 11).

Tabulating the mean concentration of the top ten phytoplankton species sampled fortnightly during the six weeks prior to August (Table 17) showed that during this period the bloom years 1993 and 1994 had more species in common than other years. Eight of the top ten species were common to both years with six of the eight common species represent non-diatomaceous taxa. Even 2-3 months before August, there was a high degree of similarity in species for 1993 and 1994, with four species in common.

A temporally close view of the phytoplankton data (Table 18) showed that cryptophytes, prasinophytes, prymnesiophytes and dinoflagellates were the major classes represented in the eight most abundant phytoplankton species in July 1993, July 1994 and July 1997. Four of the eight top species were common to both bloom years. These species were the cryptophytes *Plagioselmis prolonga* and *Teleaulax acuta*, the prasinophyte *Pyramimonas* spp., and the dinoflagellate *Katodinium rotundatum*.

MANAGEMENT IMPLICATIONS:

There are a number of growing/ harvesting and environmental management strategies that could be implemented to reduce the adverse impact of *R.* cf *chunii* blooms on the marketability of cultured mussels from Port Phillip Bay.

The growing and harvesting strategies are:

- An early warning system could be developed to predict possible blooms of *R.* cf. *chunii*, based on salinity and phytoplankton composition criteria indicators identified in this report, and implemented by industry. Phytoplankton monitoring could also be continued to provide detailed data on future bloom events.

- Research could be commissioned to investigate methods to rapidly purify mussels of the bitter-tasting compounds on a land-based facility.

- An appropriate food technology organization could be commissioned to develop taste criteria to determine if potentially affected shellfish are bitter and therefore unmarketable and an independent professional tasting panel could be established to more objectively determine when harvesting should resume after a bloom event.

- The mussel culture industry should consider the establishment of a land-based facility so that mussels can be harvested and processed (with value adding) in June and July, before *R.* cf. *chunii* blooms occur, or during any month in non-bloom years.

Environmental management strategies are:

- As a suggested precautionary measure and until the relative importance of storm-generated sediment resuspension is known, dredging for channel-maintenance, beach renourishment or any other purpose should be limited during June to September near any aquaculture zone, and particularly in the Geelong Arm of the Bay.

- As a suggested precautionary measure (and recognising that Melbourne Water is following the Port Phillip Bay Environmental Study recommendation of 1996 in reducing 500 t of N from the effluent discharged from the WTP each year) to request Melbourne Water to minimize flows from the most southerly outfall (Murtcain drain) into Port Phillip Bay from June to September.

DISCUSSION:

Distribution and abundance of *R.* cf. *chunii* in Port Phillip Bay

Rhizosolenia cf. *chunii* is a nuisance algal species, which has caused considerable damage to the mussel culture industry in Port Phillip Bay. It not only causes shellfish feeding on the algae to become intensely bitter and therefore unmarketable, but it can also cause damage and mortality of the shellfish (Parry *et al.* 1989). *Rhizosolenia* cf. *chunii* appears to be the only diatom species in the world known to cause bitter taste in shellfish, so no comparative information is available for any other species. Bitter/ hot peppery tasting mussels were found in New Zealand in 1992, but the possible cause was one of two species of raphidophyte flagellates, *Fibrocapsa japonica* or *Heterosigma akashiwo* (MacKenzie *et al.* 1992).

Parry *et al.* (1989) presented strong circumstantial evidence to show that the 'bitter taste' event in September- October 1987 in Port Phillip Bay was caused by *R.* cf. *chunii*, however phytoplankton sampling on this occasion was initiated after the bloom had developed in response to reports of bitter tasting shellfish. The subsequent blooms of *R.* cf. *chunii* in 1993 and 1994, again coinciding with reports of bitter-tasting mussels, confirmed that *R.* cf. *chunii* was indeed the causative organism. The blooms in 1993 and 1994 were less intense than in 1987, and consequently some mussels were sold after several months of recovery following the disappearance of the harmful algae.

We now know much about the temporal and spatial distribution of *R.* cf. *chunii*. Cells usually appear first in June and July each year and blooms (when they occur) have always started in early August and crash in September or early October. Some cells have been observed at other times of the year, e.g. March 1994 and December 1992, but cell numbers have always been very low.

Apart from the large blooms of *R.* cf. *chunii* in 1987, 1993 and 1994, there is anecdotal evidence to show that a bitter taste was detected in cultured mussels from Port Phillip Bay in 1975 (Parry *et al.* 1989). It is probable that *R.* cf. *chunii* was also the causative organism on this occasion. Two small blooms were detected in the inner harbour of Corio Bay in August 1991 (max. 78,000 cells L^{-1}) and August-October 1992 (max. 140,000 cells L^{-1}), but on both occasions the blooms did not spread into the Geelong Arm and the rest of Port Phillip Bay. More recently, a further widespread bloom was observed in August-September 1997; this bloom was again associated with bitter tasting shellfish.

A comprehensive study of the phytoplankton composition, distribution and abundance in Port Phillip Bay from March 1990 to February 1995 was conducted by Arnott *et al.* (1997). Based on 884 samples taken over the five-year period, these authors found that *R.* cf. *chunii* was the 60th most frequently occurring species or taxa. However, it was the 18th species in order of abundance (including absences) with a mean concentration of 9,800 cells L^{-1}. This shows that it was a very abundant species whenever it appeared. When mean species abundances, excluding absences, were examined, the relative dominance of *R.* cf. *chunii* was further revealed. *Rhizosolenia* cf. *chunii* was then the third most abundant species with a mean concentration of 207,000 cells L^{-1} at the same seven sites investigated in this report. An examination of algal blooms in Port Phillip Bay

during the same five-year period is given by Magro *et al.* (1996).

High cell concentrations of *R.* cf. *chunii* were recorded during the major bloom events. Parry *et al.* (1989) found a maximum concentration of 483,000 cells L^{-1} at Beaumaris on 18 September 1987, but earlier concentrations of 750,000 cells L^{-1} at Clifton Springs and 633,000 cells L^{-1} at Beaumaris were detected on 4 September and 7 September 1987 respectively (Arnott, unpublished data). In 1993 the highest cell concentrations were recorded in the Bay at the shellfish growing areas of Clifton Springs (660,000 cells L^{-1}) and Point Richards (310,000 cells L^{-1}) in the Geelong Arm of the Bay, and at Beaumaris (380,000 cells L^{-1}) in the northeast. Highest concentrations in 1994 were found at Werribee (1,240,000 cells L^{-1}) and Williamstown (1,200,000 cells L^{-1}) in the west and north of the Bay, although the maximum concentrations at Clifton Springs (590,000 cells L^{-1}) and Beaumaris (800,000 cells L^{-1}) were also very high. Concentrations in Corio Bay in 1993 and 1994, 250,000 and 500,000 cells L^{-1} respectively, were also much higher than in the two small blooms there in 1991 and 1992. Concentrations in the 1997 bloom were much lower than in 1987, 1993 and 1994.

The major blooms of *R.* cf. *chunii* have all developed first in the Geelong Arm of the Bay, although blooms have also developed independently in Corio Bay. From the Geelong Arm the blooms have spread in a clockwise (and possibly easterly) direction around the Bay and take about 2-4 weeks to reach sites in the north and east. Despite the blooms at Beaumaris occurring later than those at Clifton Springs, cell concentrations at this site have been high. However, the duration of exposure at Beaumaris has usually been much shorter. Dromana, on the eastern coastline, has not been affected as badly as the Clifton Springs and the nearby Point Richards shellfish growing areas, and the cultured mussels at this site have always been marketable. Maximum concentrations at Dromana were only 59,000 and 32,000 cells L^{-1} in 1993 and 1994 respectively. Cultured mussels from the Grassy Point (Portarlington) shellfish growing area have also been less affected compared to those from Clifton Springs and Point Richards, and although they have become bitter, harvesting there has been less disrupted. The concentration of *R.* cf. *chunii* at Grassy Point during bloom years has also been more variable than at other sites, mainly because the water exchanges with that from Bass Strait on each tidal cycle. *Rhizosolenia* cf. *chunii* has not bloomed in southern Port Phillip Bay to date, although low numbers of cells have been observed. Low cell concentrations have also been found at Flinders in neighbouring Western Port.

Dynamics of *R.* cf. *chunii* blooms in Port Phillip Bay

Many diatom species form cysts (or resting spores), which are an important part of their life cycle. Gran (1912) was the first to suggest that cyst formation is initiated by unfavourable growth conditions in the water column, and that after formation the cysts sink to the bottom where they can remain viable in the sediments. If the cysts are later resuspended by turbulent mixing, and environmental conditions are again favourable, the cysts can quickly germinate in vast numbers and give rise to a further bloom. Cysts, therefore, are important seed banks for population maintenance.

Cyst formation is more prevalent among marine diatom species than freshwater species,

and especially species that form large blooms (Garrison 1981). A number of species of the genus *Rhizosolenia* are known to form cysts (Sundstrom 1986), and it had been assumed by the present authors that *R.* cf. *chunii* probably also produced cysts. However, it was only recently that the Port Phillip Bay population of *R.* cf. *chunii* was observed, in the laboratory, to form cysts (van de Meene 1998). We now know, therefore, that the essential elements of the population dynamics of *R.* cf. *chunii* follow Gran's (1912) basic outline.

Why do blooms of R. cf. chunii occur only in late winter and early spring?

Strong winds are initially required to disturb the bottom sediments and resuspend the algal cysts into the water column. In Port Phillip Bay it is likely that some cysts of *R.* cf. *chunii* are continuously being resuspended into the water column following windy periods, and then sinking back to the bottom sediments, throughout the year. However, cyst germination can only occur at that time of the year when the environmental conditions are favourable for bloom development (Karentz and Smayda 1984).

Light is the most important trigger for cyst germination (Hollibaugh *et al.* 1981), and cysts can break dormancy in 24-36 hours in high light conditions (Hollibaugh *et al.* 1981, French and Hargraves 1980, Sicko-Goad *et al.* 1986). Photoperiod, in addition to light intensity, is an important germination trigger (Hobson 1981, Eilertsen *et al.* 1995). Temperature can affect the time it takes a spore to germinate, but temperature alone apparently does not initiate cyst germination (von Stosch and Fecher 1979). Germination is not influenced by nutrient concentrations because nutrient uptake and cell division does not occur until after excystment (Hollibaugh *et al.* 1981, Smetacek 1985).

Similar species successions have been observed at certain coastal localities with different environmental regimes, and blooms have developed at the same time of the year under highly variable environmental conditions at a single locality (Eilertson *et al.* 1995, McQuoid 1995). Ziemann *et al.* (1991), for example, observed only a 3-5 day interannual variability in the timing of the spring bloom in Auke Bay in Alaska. These findings indicate that strong seasonal cues can be an important stimulus to bloom development. Photoperiod alone (Eilertson *et al.* 1995) or in association with temperature (McQuoid and Hobson 1995) can control the germination of cysts and the growth of subsequent vegetative cells of some species.

Blooms of *R.* cf. *chunii* in Port Phillip Bay have always developed in early August. In 1993 and 1994 concentrations of 47,000 cells L^{-1} and 40,000 cells L^{-1} were recorded at Clifton Springs on 4 August and 3 August, respectively. Although some cells were observed during previous weeks in both years, cell concentrations during this earlier period were always < 5,000 cells L^{-1}. The timing of the 1997 bloom was similar, but a rapid increase in number did not happen until mid-August. Recorded concentrations at Point Richards (about 2-3 km east of Clifton Springs) were as follows: 30 July (20,000 cells L^{-1}), 5 August (11,000 cells L^{-1}), 14 August (18,000 cells L^{-1}) and 19 August (65,000 cells L^{-1}). In 1987 phytoplankton sampling only started in the first week of September, by which time a concentration of 750,000 cells L^{-1} had already developed at Clifton Springs. However, reports of bitter tasting mussels from the Geelong Arm in mid to late August

indicate that the bloom in 1987 must have been initiated at least by mid-August.

The above data reveal a strong interannual synchronization of *R.* cf. *chunii* blooms in the Geelong Arm of Port Phillip Bay, and indicate that a strong seasonal cue must have been operating to trigger cyst germination. We suggest that this cue is likely to be the photoperiod, probably acting in combination with temperature. August is about six weeks past the winter solstice and hence daylength is increasing at this time, while water temperatures are at their annual minimum in early August. Eilertson *et al.* (1995) found that some spring-blooming phytoplankton species from Norwegian waters failed to grow in the laboratory when exposed to daylengths shorter than 13 hours. A similar response is likely to be found with *R.* cf. *chunii*, although laboratory experiments to assess the influence of photoperiod and temperature have yet to be conducted. Based on field data, it is likely that *R.* cf. *chunii* cysts do not germinate unless exposed to daylengths > 10 h; 10-h daylengths first occur in winter in Melbourne on 26 July. Eilertson *et al.* (1995) also found that cells of other common species like *Skeletonema costatum* grew irrespective of daylength. *Skeletonema costatum* is present throughout the year in Port Phillip Bay, where it is the dominant species (Arnott *et al.* 1997), and its growth is also unlikely to be curtailed by daylength in local waters.

Why do blooms originate in the Geelong Arm of Port Phillip Bay?

Before a bloom of *R.* cf. *chunii* can develop, strong winds are required to disturb the bottom sediments and resuspend the cysts into the water column. However, the strength of the wind required to create the necessary vertical turbulence depends on the depth of water. Water depth in the main central basin of Port Phillip Bay is 10 m or greater, with much of this area 15 m or deeper. However, the Geelong Arm of the Bay is relatively shallow with water depths less than 10 m. Cysts of *R.* cf. *chunii* in the Geelong Arm would therefore be more likely to be resuspended, and resuspended more often, than cysts in other areas of the Bay. Winds from the northeast and southwest have the greatest fetch in the Geelong Arm, and these winds are more likely to resuspend cysts from the bottom sediments than other winds.

It is also likely that channel-maintenance and other types of dredging of sediments can resuspend cysts into the water column.

Diatom cysts and vegetative cells have no means of self-propulsion and therefore require some ongoing minimum vertical turbulence to maintain their position in the water column (Harris 1986). Wind-driven turbulence causes the cells to travel between surface and bottom waters, thus exposing them to variations in light intensity and potentially to variations in nutrient concentration. In most of Port Phillip Bay the water column is usually well mixed, and there is rarely a significant difference in nutrient concentration with depth except offshore from the WTP and in Hobsons Bay (Longmore *et al.* 1996). The water column is also mixed often enough by wind, especially in the Geelong Arm, to prevent light-limitation of algal growth.

The considerable increase in nutrient loads discharged from the Western Treatment Plant (WTP) in winter, and the proximity of the WTP to the Geelong Arm, is a further major

reason for the *R.* cf. *chunii* blooms first developing in the Geelong Arm of Port Phillip Bay. Data presented in this report shows that the discharge flow of the WTP is highest in June and July, and the NH_4 load to the Bay is significantly higher in winter and early spring (Table 5). The concentrations of NH_4, NO_2 and NO_3 are also significantly higher in June and July at Clifton Springs (Table 8). Nutrient concentrations in the south east of the Bay, on the other hand, are consistently lower than those in the north and west (Longmore *et al.* 1996), and this may explain why *R.* cf. *chunii* blooms have not developed or spread to this area of the Bay.

An increase in phytoplankton biomass by up to 5 $\mu g\ L^{-1}$ of chlorophyll was observed over two weeks during *R.* cf. *chunii* blooms. Using the Redfield ratio, we can estimate the nutritional demand of such planktonic growth. A chlorophyll production rate of 5 $\mu g\ L^{-1}$ per fortnight over the volume of the Geelong Arm would require about 21 tonnes N and 42 tonnes Si. The WTP discharges about 170 tonnes N (Murray 1994) and 49 tonnes Si each fortnight in winter. In contrast, fortnightly loads in summer are about 30 tonnes N and 20 tonnes Si. It is therefore probable that the WTP cannot supply enough N and Si for the development of a *R.* cf. *chunii* bloom except in winter and early spring, and even then the silicate demand of the bloom would closely match the supply. Furthermore, the size of the bloom may at times be limited by the amount of available SiO_4. Low concentrations of silicate were found in the water column in September of both 1993 and 1994 (Fig. 5a), indicating that the *R.* cf. *chunii* blooms in these two years probably crashed because of a lack of this nutrient.

Why do blooms of R. cf. chunii develop only in some years?

Whether a bloom of *R.* cf. *chunii* develops in any particular year depends on many factors. The factors investigated in this report include wind-driven turbulence, water temperature and salinity, and nutrient and light availability.

The wind pattern in mid to late-July and in August varied markedly between years, but there was no evidence to suggest that bloom years were different to the rest. Furthermore, with one exception, there were one or more windy days in the last two weeks of July each year. Thus, it is likely that cysts would have been present annually in the water column in the Geelong Arm during the most critical period. In 1986 there was no wind >5 $m\ s^{-1}$ between 9 July and 19 August.

As for wind, bloom and non-bloom years could not be separated by water temperature and light availability data in July-August. Measurements of PAR at Clifton Springs indicated that sufficient light was usually present in the Geelong Arm to enable phytoplankton growth in bottom waters.

Ambient nutrient concentrations (and their ratios) are critical factors for phytoplankton growth. During the years 1990-1995 the seasonal discharge of nutrients from the WTP was fairly consistent and therefore similar in both bloom and non-bloom years. The WTP nutrient load was high in June- July 1995 (Fig. 4a), as in 1993 and 1994, but a bloom of *R.* cf. *chunii* failed to develop in that year. However, N concentrations at Clifton Springs (especially NH_4) were very low for the whole of 1995 (Fig. 5a), and the N:Si ratio was

less than 1 (Fig. 5b), indicating N limitation of diatom growth. It thus appears that the N discharged from the WTP in the winter of 1995 did not reach the Geelong Arm.

The most significant feature of the bloom years 1975, 1987, 1993 and 1994 was the low mean salinity. Salinity has been measured in Port Phillip Bay in a series of non-continuous programs since 1968 (MMBW & FWD 1973; EPA 1979; Cowdell *et al.* 1985; Mickelson 1990; Longmore *et al.* 1990; and Longmore *et al.* 1996). Salinity in the Geelong Arm in 1993 and 1994 was significantly lower than in 1990-1992 and 1995 and about one unit lower than average. Close inspection of all the data reveals that of the 27 years from 1968-1995, salinity data were available for winter in 18 years, and low salinity water in the winter of 1975 can also be inferred from the very low salinity recorded in October-December 1975. Of the 19 years for which we have data, salinity fell below 34.5 in July and/or August in five years (1975, 1987, 1993, 1994, 1995), and blooms of *R.* cf. *chunii* cells occurred in the first four of these years (Table 19). The salinity remained low for several months in 1975, 1993 and 1994, but only one month in 1987.

Additional salinity data for 1996 and 1997 (Longmore, unpubl. data) can also be assessed. The salinity at Clifton Springs was < 34.5 in January 1996, and again from 2 July-31 December 1996, but no salinity < 34.5 occurred in 1997 (Table 19). A bloom of *R.* cf. *chunii* did not develop in 1996 but did develop in August-September 1997 (Table 19); the reverse outcome may have been expected based on the salinity data.

Three substantial reductions in salinity occurred at Clifton Springs during the period from 1990-1997 (Fig. 12). Minimum salinities were recorded on 2 February 1993 (33.42), 10 November 1993 and 7 January 1994 (both 33.27), and 24 September 1996 (33.46). A further smaller decrease to 34.04 was also recorded on 22 November 1995. Blooms of *R.* cf. *chunii* occurred in August-September after all three major salinity reductions, despite the 6-11 month delay in time between the salinity minimum and bloom initiation.

We do not yet know why low salinity water was associated with bloom development in four of the six years when salinities were < 34.5 in July-August (years 1995 and 1996 were the exceptions), nor why blooms should develop in July-August following salinities <33.75 occurring within the previous 11 months. Salinity *per se*, or silicate or some trace element or micronutrient introduced in the freshwater runoff, may be the important factor. Caffrey (1995) observed that terrestrial-sourced inputs could increase primary production in the receiving water. *Gymnodinium catenatum* blooms in Tasmanian waters only develop within a seasonal temperature window, triggered by a rainfall event which is followed by calm periods (wind speed <5 m s^{-1}) for five days or more (Hallegraeff *et al.* 1995). If some growth factor was partially responsible for *R.* cf. *chunii* blooms in Port Phillip Bay, it would have had to remain in Bay waters for some considerable time.

The phytoplankton analyses in this report revealed that the community composition in the weeks before August of 1993 and 1994 was quite similar and different to that in non-bloom years. Perhaps the most important finding was that diatom concentrations were very low in June and July of 1993 and 1994, but relatively more abundant in these months in other years. This result can readily be seen in Figure 13, after Arnott *et al.* (1997), who found that diatoms formed only 1.2% and 0% of phytoplankton numbers in July 1993 and 1994 respectively. In comparison, diatom relative abundance in July 1990-1992 ranged

between 16.2% and 45.5%. Cryptophytes (20.7%) and prasinophytes (63.1%) were the dominant classes in July 1993, while cryptophytes (67.7%) were dominant in July 1994.

During July 1997, another bloom year, the diatom relative abundance was very low at the start and middle of the month, i.e. 3.4% on 1 July and 12.3 % on 17 July, but increased rapidly to 78.3% by 30 July 1997 (Arnott, unpubl. data). *Rhizosolenia.* cf. *chunii* was still a relatively minor species on 30 July 1997, but bloomed soon after in early August. Prymnesiophytes, cryptophytes and dinoflagellates were the dominant classes (in that order) on 1 July 1997. Four of the eight most abundant species present at Clifton Springs in July 1997, namely *P. prolonga*, *T. acuta*, *Pyramimonas* spp. and *K. rotundatum*, were also relatively abundant in both 1993 and 1994 (Table 18). The dominance of classes and species other than diatoms in June and July may create, or may simply reflect, conditions suitable for the later development of a *R.* cf. *chunii* bloom in August.

We are unable to say why a bloom of *R.* cf. *chunii* initiates, apart from an appreciation of the factors of temperature, light, wind speed and nutrients availability. The blooms do not appear to be consistently associated with large-scale climatic events such as *El Nino*. A strong *El Nino* event occurred during 1982-83 but there was no evidence, anecdotal or otherwise, to indicate that a *R.* cf. *chunii* bloom occurred in these years. A much weaker event occurred in the bloom year 1987, and even weaker climate perturbations in the bloom years of 1993 and 1994. However, in 1975 a *La Nina* event, the opposite of *El Nino*, occurred. The *La Nina* developed when seawater temperatures off the western coast of South American were colder than usual (Harvey Stern, Bureau of Meteorology, Melbourne, pers. comm). As previously stated, there is good anecdotal evidence to suggest that a bloom of *R.* cf. *chunii* occurred during 1975.

However, we have recognised, within the temporal limitations of the database, two strong indicators. These are a salinity depression happening during the previous summer of a bloom year and the relative species abundance of the phytoplankton community 4 - 6 weeks before August.

Management implications

There are a number of growing/ harvesting and environmental management strategies that could be implemented to reduce the adverse impact of *R.* cf *chunii* blooms on the marketability of cultured mussels from Port Phillip Bay.

Likely growing/harvesting strategies that could be developed are: An early warning system to predict possible blooms of *R.* cf. *chunii*, based on salinity and phytoplankton composition criteria indicators identified in this report. Phytoplankton monitoring could also be continued to provide detailed data on future bloom events. Research could be commissioned to investigate methods to rapidly purify mussels of the bitter-tasting compounds on a land-based facility. An appropriate food technology organization could be commissioned to develop taste criteria to determine if potentially affected shellfish are bitter and therefore unmarketable and an independent professional tasting panel could be established to more objectively determine when harvesting should resume after a bloom event. A suitable land-based facility could be established so that mussels can be harvested and processed (with value adding) in June and July, before *R.* cf. *chunii* blooms occur, or

during any month in non-bloom years.

Suggested environmental management strategies as precautionary measures are: Until the relative importance of storm-generated sediment resuspension is known, dredging for channel-maintenance, beach renourishment or any other purpose should be limited during June to September near any aquaculture zone, and particularly in the Geelong Arm of the Bay. To request Melbourne Water to minimize flows from the most southerly outfall (Murtcain drain) into Port Phillip Bay from June to September, while recognising that Melbourne Water is following the Port Phillip Bay Environmental Study recommendation of 1996 in reducing 500 t of N from the effluent discharged from the WTP each year.

BENEFITS:

This project will be of benefit to the mussel culture industry in Port Phillip Bay and particularly the growers operating in the Geelong Arm. Predictions of the likely occurrence of *R.* cf. *chunii* blooms can aid mussel farmers in reducing the likelihood of crop losses by either harvesting mussels earlier or shifting some or all of their crop to areas less likely to be affected by blooms. Other beneficiaries may include consumers of farmed mussels and potential harvesters of other bivalve aquaculture or wild stock.

FURTHER DEVELOPMENT:

Growing/ Harvesting Strategies

- *An early warning system to predict possible blooms of* R. *cf.* chunii *should be developed, based on salinity and phytoplankton composition criteria identified in this report, and implemented by industry. Phytoplankton monitoring should also be continued to provide detailed data on future bloom events.*

- *Research should be commissioned to investigate methods to rapidly purify mussels of the bitter-tasting compounds on a land-based facility.*

- *An appropriate food technology organization should be commissioned to develop taste criteria to determine if potentially affected shellfish are bitter and therefore unmarketable, and to establish an independent professional tasting panel to more objectively determine when harvesting should resume after a bloom event.*

- *The mussel culture industry should consider the establishment of a land-based facility so that mussels can be harvested and processed (with value adding) in June and July, before* R. *cf.* chunii *blooms occur, or during any month in non-bloom years.*

Environmental Management

- *As a suggested precautionary measure and until the relative importance of storm-generated sediment resuspension is known, dredging for channel-maintenance, beach renourishment or any other purpose should be limited during June to September near any aquaculture zone, and particularly in the Geelong Arm of the Bay.*

- *As a suggested precautionary measure (and recognising that Melbourne Water is following the Port Phillip Bay Environmental Study recommendation of 1996 in reducing 500 t of N from the effluent discharged from the WTP each year) to request Melbourne Water to minimize flows from the most southerly outfall (Murtcain drain) into Port Phillip Bay from June to September.*

Immediate Research Needs

- *Research should be commissioned to isolate the unknown compound(s) responsible for the bitter taste in shellfish, and to develop an analytical method to measure the concentration of the bitter-tasting compound(s) or associated 'biomarkers' present in shellfish tissue as a determinant for marketability.*

CONCLUSION:

Based on knowledge gained from the appearance of *R.* cf. *chunii* blooms in August-September of 1987, 1993, 1994 and 1997, we can now hypothesize about the environmental conditions required for bloom development. Several factors, acting in combination, seem important. Some wind stress is initially required in late July or early August to resuspend cysts of *R.* cf. *chunii* into the water column. A specific photoperiod (a daylength of about ten hours occurring at a time when daylength is increasing), combined with low water temperature (minimum annual temperatures occur in early August), are strong seasonal cues operating to trigger cyst germination and bloom development. Blooms originate in the Geelong Arm of the Bay because of its shallow depth (enabling wind-driven re-suspension of cysts) and supply of nutrients including N and Si (arising from the considerably increased nutrient loads discharged from the Western Treatment Plant in winter). A slightly reduced salinity, either salinity *per se* or a growth factor introduced via freshwater runoff, also appears a necessary requirement and is probably the main factor responsible for blooms appearing only in some years.

The observations within the body of this report can be used to develop a predictive model for *R.* cf. *chunii* blooms. We may predict that blooms will develop whenever salinity falls below 34.5 in July-August. This criterion would have successfully predicted four of the five blooms observed so far, but would have predicted blooms in 1995 and 1996 when none occurred. In addition, this same criterion would have correctly predicted the absence of blooms in 14 years, and incorrectly predict no bloom in 1997 when a bloom was observed. The probability of a false alarm is therefore fairly low, although the predictions for the last three years would have been wrong if based solely on the 34.5 salinity criterion.

We can also predict that blooms will develop when salinity decreases to <33.75 sometime during the 11 months before August, the month when all past blooms have developed. Use of this criterion would have successfully predicted the last three bloom events (1993, 1994 and 1997), and would not have predicted any false alarms. However, this criterion is based on only eight years of data and hence will need to be followed in future years. In the interim, it is suggested that both salinity criteria should be utilized for future predictions. A low relative abundance of diatoms in June and July, combined with a high relative abundance of the four non-diatom species *P. prolonga*, *T. acuta*, *Pyramimonas* spp. and *K. rotundatum*, may also indicate a greater likelihood of a *R.* cf. *chunii* bloom developing in August of that year.

Salinity can be measured frequently, accurately and relatively cheaply, making it a good candidate for an early warning system. Furthermore, it can be measured remotely, leading to the possibility of automatic salinity reports, or reporting on request, to a land base facility. Whenever it occurs, salinity depression is a Bay-wide phenomenon, so that measurement at one site should be sufficient to make a Bay-wide prediction. Given the dynamics of bloom growth, there should be a period of at least 1-2 weeks between any marked decrease in salinity and the development of a *R.* cf. *chunii* bloom, and a further week or so before any bitter taste is detected in shellfish. During this time mussel farmers may be able to harvest or shift some or their entire crop from areas likely to be affected. In addition, if bay-wide salinities are substantially reduced for several months including the

months of May or June, 2-3 months warning of a potential *R.* cf. *chunii* bloom may be given.

ACKNOWLEDGMENTS

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REFERENCES:

Anon. (1979). Methods for the chemical analysis of water and wastes. US Environmental Protection Agency, Ohio.

Arnott, G.H., Gason, A.S., Hill, D.R.A., Magro, K.L., Reilly, D.J. and Coots, A.G. (1997). Phytoplankton composition, distribution and abundance in Port Phillip Bay from March 1990 to February 1995. Port Phillip Bay Environmental Study, Technical Report No. 40. CSIRO, Canberra, Australia.

Belbin, L. (1990). The analysis of pattern in bio-survey data. In *Nature Conservation: Cost Effective Biological Surveys and Data Analysis,* C. R. Margutes and M. P. Austin (Eds.).

Bendschneider, K. and Robinson, R.J. (1952). A new spectrophotometric method for the determination of nitrite in seawater. *J. Mar. Res.* 11: 87-96.

Caffrey, J. M. (1995). Spatial and seasonal patterns in sediment nitrogen remineralization and ammonium concentrations in San Fransisco Bay, California. *Estuaries* 18: 219-233.

Cowdell, R.A., Gibbs, C.F., Longmore, A.R. and Theodoropolous, T. (1985). Tabulation of Port Phillip Bay water quality data between June 1980 and July 1984. Internal Report No. 98, Marine Science Laboratories, Queenscliff, Victoria.

Eilertsen, H. C., Sandberg, S. and Tollefsen, H. (1995). Photoperiodic control of diatom spore growth. *Mar. Ecol. Prog. Ser.* 116: 303-7.

EPA (1979). Port Phillip Bay water quality monitoring programme. Report No. 93/79, Environment Protection Authority, Melbourne.

Fisher, N.S. and Cowdell, R.A. (1982). Growth of marine planktonic diatoms on inorganic and organic nitrogen. *Mar. Biol.* 72: 147-55.

French, F.W. and Hargraves, P.E. (1980). Physiological characteristics of plankton diatom resting spores. *Mar. Biol. Lett.* 1: 185-95.

Garrison, D.L. (1981). Monterey Bay phytoplankton. I. Seasonal cycles of phytoplankton assemblages. *J. Plankton Res.* 3: 137-56.

Gran, H.H. (1912). Pelagic plant life. In *The Depths of the Ocean*, J. Murray and J. Hort (Eds.), pp. 307-386. Macmillan Press, London.

Hallegraeff, G. M., McCausland, M. A. and Brown, R. K. (1995). Early warning of toxic dinoflagellate blooms of *Gymnodinium catenatum* in southern Tasmanian waters. *J. Plankton Res.* 17: 1163-76.

Harris, G. P. (1986). Phytoplankton Ecology. Structure, Function and Fluctuation. Chapman and Hall Ltd., London.

Heath, M.R., Richardson, K. and Kiorboe, T. (1990). Optical assessment of phytoplankton nutrient depletion. *J. Plankton Res.* 12: 381-96.

Hobson, L.A. (1981). Seasonal variations in maximum photosynthetic rates of phytoplankton in Saanich Inlet, Vancouver Island, British Columbia. *J. Exp. Mar. Biol. Ecol.* 52: 1-13.

Hollibaugh, J.T., Siebert, D.R. and Thomas, W.H. (1981). Observations on the survival and germination of resting spores of three *Chaetoceros* (Bacillariophyceae) species. *J. Phycol.* 17: 1-9.

Jeffrey, S.W. and Humphrey, G.F. (1975). New spectrophotometric equations for determining chlorophylls a, b, c_1 and c_2 in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanzen* 167: 191-4.

Karentz, D. and Smayda, T. (1984). Temperature and seasonal occurrence patterns of 30 dominant species in Narragansett Bay over a 22 year period (1959-1980). *Mar. Ecol. Prog. Ser.* 18: 277-93.

Koroleff, F. (1972). Determination of reactive silicate. In *New Baltic Manual with Methods for Sampling and Analyses of Physical, Chemical and Biological Parameters*, S.R Carlberg (Ed.), pp 87-90. Cooperative Research Report, Series A, No. 29. International Council for the Exploration of the Sea, Charlottenlund Slot, KD-2920 Charlottenlund, Denmark.

Longmore, A.R., Cowdell, R.A. and Flint, R. (1996). Nutrient status of the water in Port Phillip Bay. Port Phillip Bay Environmental Study, Technical Report No. 24. CSIRO, Canberra, Australia.

Longmore, A.R., Cowdell, R.A. and Gibbs, C.F. (1990). Monitoring of water quality in Port Phillip Bay, 1985-86. Scientific Report Series No. 89/003, Environment Protection Authority, Melbourne.

McQuoid, M.R. (1995). Seasonal succession and interannual variability of diatoms (Bacillariophyceae) from Saanich Inlet, British Columbia, in relation to seasonal and climatic factors. Ph. D. dissertation, University of Victoria, Victoria, 294 pp.

McQuoid, M.R. and Hobson, L.A. (1995). Importance of resting stages in diatom seasonal succession. *J. Phycol.* 31: 44-50.

McQuoid, M.R. and Hobson, L.A. (1996). Diatom resting stages. *J. Phycol.* 32: 889-902.

MacKenzie, L., Rhodes, L. and White, D. (1992). An investigation of algal biotoxins, in plankton and mussels from the Coromandel area, 8-10 November 1992. A Report to the Ministry of Agriculture and Fisheries, New Zealand. Cawthron Institute, Nelson, New Zealand.

Magro, K.L., Arnott, G.H. and Hill, D.R.A. (1996). Algal blooms in Port Phillip Bay from March 1990 to February 1995: temporal and spatial distribution and dominant species. Port Phillip Bay Environmental Study, Technical Report No. 27. CSIRO, Canberra, Australia.

MMBW & FWD (1973). Environmental Study of Port Phillip Bay. Report on Phase One, 1968-71. Melbourne and Metropolitan Board of Works and Fisheries and Wildlife Department, Melbourne.

Mickelson, M.J. (1990). Dissolved oxygen in bottom waters of Port Phillip Bay. Environmental Services Series No. 90/010. Board of Works, Melbourne.

Morris, A.W. and Riley, J.P. (1963). The determination of nitrate in seawater. *Anal. chim. Acta* 29: 272-9.

Murphy, J. and Riley, J.P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Anal. chim. Acta* 27: 31-6.

Murray, A.G. (1994). Western treatment plant outputs to Port Phillip Bay. Port Phillip Bay Environmental Study, Technical Report No. 15. CSIRO, Canberra, Australia.

Nicholls, K.H. (1975). A single digestion procedure for rapid manual determination of Kjeldahl nitrogen and total phosphorus in natural waters. *Anal. chim. Acta* 76: 208-12.

Parry, G.D., Langdon, J.S. and Huisman, J.M. (1989). Toxic effects of a bloom of the diatom *Rhizosolenia chunii* on shellfish in Port Phillip Bay, southeastern Australia. *Mar. Biol.* 102: 25-41.

Redfield, A.C., Ketchum, B.H. and Richards, F.A. (1963). The influence of organisms on the composition of sea water. In *The Sea. Vol. 2.*, M.N. Hill (Ed.), pp 26-77. Interscience, New York.

SAS Institute Inc. (1990). SAS Language: Reference, Version 6, First Edition. SAS Institute Inc., Cary, North Carolina.

Sicko-Goad, L., Stoermer, E.F. and Fahnenstiel, G. (1986). Rejuvenation of *Melosira granulata* (Bacillariophyceae) resting cells from the anoxic sediments of Douglas Lake, Michigan. I. Light microscopy and ^{14}C uptake. *J. Phycol.* 22: 22-8.

Smetacek, V.S. (1985). Role of sinking in diatom life-history cycles: ecological, evolutionary, and geological significance. *Mar. Biol.* 84: 239-51.

Solorzano, L. (1969). Determination of ammonia in natural waters by the phenol hypochlorite method. *Limnol. Oceanogr.* 14: 799-801.

Strickland, J.D.H. and Parsons, T.R. (1972). A Practical Handbook of Seawater Analysis. Second Edition. Bulletin No. 267, Fisheries Research Board of Canada, Ottawa.

Sundstrom, B.G. (1986). The marine diatom genus *Rhizosolenia*. Ph.D. dissertation, Lund University, Lund, Sweden, 117 pp.& 39 pls.

Technicon (1972). Nitrate and nitrite in seawater. Industrial Method No. 158-71W. Technicon Instrument Corporation, Tarrytown, New York.

Technicon (1973). Orthophosphate in water and seawater. Industrial Method No. 155-71W. Technicon Instrument Corporation, Tarrytown, New York.

Technicon (1973a). Ammonia in water and seawater. Industrial Method No. 154-71W. Technicon Instrument Corporation, Tarrytown, New York.

Technicon (1973b). Silicates in water and seawater. Industrial Method No. 186-71W. Technicon Instrument Corporation, Tarrytown, New York.

van de Meene, A. (1998). Resting spore formation in *Rhizosolenia* cf. *chunii*. A Report to the Marine and Freshwater Resources Institute, Queenscliff, Victoria. School of Botany, University of Melbourne, Melbourne.

von Stosch, H.A. and Fecher, K. (1979). "Internal thecae" of *Eunotia soleirolii* (Bacillariophyceae) : development, structure and function as resting spores. *J. Phycol.* 15: 233-43.

Ziemann, D.A., Conquest, L.D., Olaizola, M. and Bienfang, P.K. (1991). Interannual variability in the spring phytoplankton bloom in Auke Bay, Alaska. *Mar. Biol.* 109: 321-34.

APPENDIX 1:

Intellectual Property:

No intellectual property has arisen from the research that is likely to lead to significant commercial benefits, patents or licenses. Intellectual property associated with data produced from the project will be shared equally by the Fisheries Research and Development Corporation and the Victorian Department of Natural Resources and Environment.

APPENDIX 2:

Staff:

G. H. Arnott	Principal investigator
G. J. Nicholson	Project scientist
A. R. Longmore	Co-investigator
M. I. Sporcic	Statistical advice

TABLES

Table 1: Years when *R.* cf. *chunii* was observed at any of the seven key sites in Port Phillip Bay, and the years in which it bloomed between 1987-1995.

Year	Observed	Bloomed/Bitter taste
1987	Yes	Yes
1988	No	No
1989	Yes	No
1990	No	No
1991	Yes	No
1992	Yes	No
1993	Yes	Yes
1994	Yes	Yes
1995	Yes	No

Table 2: *Rhizosolenia* cf. *chunii* abundance as a percentage of total phytoplankton abundance (mean of seven key sites) between 1987-1995.

Year	Month	*R.* cf. *chunii* abundance as percentage of total abundance
1987	Sep	97
	Oct	38
1989	Oct	4
1991*	Aug	< 1
1992*	Dec	< 1
1993	Aug	12
	Sep	11
1994	Mar	< 1
	Aug	18
	Sep	55
1995	Aug	< 1
	Sep	< 1

* Small Aug-Sept bloom only in Corio Bay inner harbour.

Table 3: Levels of significance for WTP effluent parameters, derived by ANOVA, indicating temporal scales of variability. Significant effects ($p < 0.05$) shown in bold.

Type	Flow	NH_4	NO_2	NO_3	PO_4	OrgN	TotP	TOC
Year	**0.03**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	0.16	**< 0.01**
Month	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	0.40	**< 0.01**	**< 0.01**
Year*Month	**< 0.01**	**0.04**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**0.02**	**< 0.01**

Table 4: Years which have the greatest mean value of each of the parameters associated with the WTP discharge are at the top of each column and are arranged in descending order to the least mean value for each of the parameters at the bottom of the column. The significance of the difference between the hierarchical annual mean values within each column is indicated by the capital letter under each year. Years with the same capital letters within each column have means which are not significantly different. The *R.* cf. *chunii* bloom years of 1993 and 1994 are shown in bold.

Flow	NH_4	NO_2	NO_3	PO_4	OrgN	TotP	TOC
1990	1990	1995	1995	1995	1991	1990	1990
A	A	A	A	A	A	A	A
1992	1992	1992	**1993**	**1993**	**1994**	1995	**1994**
A	A	AB	**B**	**B**	**A**	AB	**A**
1995	1991	**1993**	**1994**	**1994**	**1993**	1992	1992
AB	AB	**AB**	**B**	**B**	**A**	ABC	B
1993	**1993**	1990	1992	1990	1990	1991	1991
AB	**AB**	AB	C	B	A	BC	BC
1991	**1994**	**1994**	1990	1992	1992	**1993**	**1993**
BC	**B**	**BC**	D	B	A	**BC**	**BC**
1994	1995	1991	1991	1991	1995	**1994**	1995
C	B	C	E	C	B	**C**	C

Table 5: Months which have the greatest mean value of each of the parameters associated with the WTP discharge are at the top of each column and are arranged in descending order to the least mean value for each of the parameters at the bottom of the column. The significance of the difference between the hierarchical monthly mean values within each column is indicated by the capital letter under each month. Months with the same capital letters within each column have means which are not significantly different. June (Jun) and July (Jul) are shown in bold as these are the two months preceding August, which appears to be the favoured month for bloom initiation when it occurs.

Flow	NH_4	NO_2	NO_3	PO_4	OrgN	TotP	TOC
Jun	Sep	Dec	Aug	Sep	Jan	Sep	**Jun**
A	A	A	A	A	A	A	**A**
Jul	**Jul**	Oct	**Jul**	**Jul**	May	**Jun**	May
A	**A**	AB	**AB**	**AB**	A	**AB**	A
Sep	Aug	Jan	Sep	Aug	Nov	**Jul**	Sep
A	A	AB	ABC	AB	AB	**AB**	B
Aug	**Jun**	Aug	**Jun**	**Jun**	**Jul**	Aug	**Jul**
A	**A**	BC	**ABC**	**AB**	**AB**	AB	**B**
May	Oct	Nov	Oct	Oct	Oct	Oct	Nov
AB	B	BC	BCD	BC	AB	AB	B
Oct	May	Feb	May	May	Dec	May	Oct
AB	BC	CD	BCD	C	AB	BC	B
Nov	Nov	**Jul**	Dec	Nov	Apr	Nov	Aug
BC	C	**CD**	BCD	C	AB	CD	B
Dec	Dec	Sep	Apr	Dec	Aug	Apr	Dec
CD	D	CD	CD	D	AB	DE	B
Apr	Apr	**Jun**	Jan	Apr	Sep	Dec	Apr
CD	DE	**CDE**	CD	D	AB	DEF	BC
Jan	Jan	May	Nov	Jan	**Jun**	Jan	Mar
CD	EF	CDE	D	ED	**AB**	EF	CD
Mar	Mar	Apr	Mar	Mar	Mar	Mar	Feb
D	EF	DE	E	ED	AB	EF	D
Feb	Feb	Mar	Feb	Feb	Feb	Feb	Jan
D	F	E	E	E	B	F	D

Table 6: Levels of significance for certain chemical parameters at Clifton Springs (Site 13), derived by ANOVA indicating temporal scales of variability. Significant effects ($p < 0.05$) shown in bold.

Type	NH_4	NO_2	NO_3	PO_4	SiO_4	OrgN	TotP	TOC	PrtP	PrtN	POC
Year	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**0.04**	**<0.01**	**<0.01**
Month	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.10	0.18	**0.02**	0.92
Year*Month	**0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.09	0.15	**<0.01**	0.85

Table 7: Years which have the greatest mean value of each of the chemical parameters measured at Clifton Springs (Site 13) are at the top of each column and are arranged in descending order to the least mean value for each of the parameters at the bottom of the column. The significance of the difference between the hierarchical annual mean values within each column is indicated by the capital letter under each year. Years with the same capital letters within each column have means which are not significantly different. The *R.* cf. *chunii* bloom years of 1993 and 1994 are shown in bold.

NH_4	NO_2	NO_3	PO_4	SiO_4	OrgN	TotP	TOC	PrtP	PrtN	POC
1993	1991	1992	1992	1990	**1993**	1990	**1993**	1990	1990	1990
A	A	A	A	A	**A**	A	**A**	A	A	A
1992	1992	1991	**1993**	**1993**	1992	1992	1995	1991	1995	**1993**
A	AB	A	**A**	**B**	B	A	AB	A	A	**B**
1990	**1993**	**1993**	1990	**1994**	**1994**	**1993**	**1994**	1995	1991	1991
AB	**BC**	**AB**	A	**B**	**B**	**A**	**B**	AB	AB	B
1991	1990	**1994**	1991	1995	1990	1991	1992	**1993**	**1993**	1992
AB	BC	**ABC**	B	B	B	B	B	**B**	**AB**	BC
1994	**1994**	1990	1995	1992	1991	1995	1990	1992	1992	1995
B	**BC**	BC	B	BC	B	B	B	B	B	BC
1995	1995	1991	**1994**	1991	1995	**1994**	1991	**1994**	**1994**	**1994**
C	C	C	**B**	C	B	**B**	B	**B**	**B**	**C**

Table 8: Months which have the greatest mean value of each of the chemical parameters measured at Clifton Springs (Site 13) are at the top of each column and are arranged in descending order to the least mean value for each of the parameters at the bottom of the column. The significance of the difference between the hierarchical monthly mean values within each column is indicated by the capital letter under each month. Months with the same capital letters within each column have means which are not significantly different. June (Jun) and July (Jul) are shown in bold as these are the two months preceding August, which appears to be the favoured month for bloom initiation when it occurs.

NH_4	NO_2	NO_3	PO_4	SiO_4	OrgN	TotP	TOC	PrtP	PrtN	POC
Jul	**Jul**	**Jul**	**Jul**	Apr	**Jul**	**Jul**	Aug	Sep	**Jul**	May
A	A	A	A	A	A	A	A	A	A	A
Jun	**Jun**	**Jun**	Aug	May	**Jun**	Aug	**Jul**	Dec	Dec	**Jul**
A	B	B	AB	A	AB	AB	AB	AB	AB	A
Dec	Aug	Aug	May	Mar	Aug	May	**Jun**	Nov	Aug	Sep
B	C	C	BC	AB	ABC	AB	AB	AB	AB	A
Aug	May	May	**Jun**	Jan	Feb	**Jun**	Sep	**Jul**	Nov	Dec
B	C	C	BC	AB	BCD	BC	AB	AB	ABC	A
May	Dec	Dec	Sep	Feb	May	Sep	May	Feb	Sep	Feb
B	C	C	CD	AB	BCD	BCD	AB	AB	ABCD	A
Oct	Jan	Mar	Apr	**Jul**	Dec	Oct	Apr	Aug	Jan	Aug
B	C	C	DE	AB	BCD	CDE	AB	AB	ABCD	A
Mar	Feb	Sep	Oct	**Jun**	Apr	Apr	Feb	Jan	Mar	Mar
B	C	C	EF	ABC	CD	DE	AB	AB	ABCD	A
Apr	Apr	Apr	Mar	Dec	Mar	Nov	Dec	Mar	Feb	Jan
B	C	C	FG	BC	CD	EF	AB	AB	ABCD	A
Sep	Oct	Feb	Nov	Nov	Oct	Mar	Mar	Apr	Apr	Nov
B	C	C	FGH	BC	CD	EF	AB	AB	BCD	A
Jan	Mar	Oct	Dec	Oct	Nov	Feb	Oct	**Jun**	May	Oct
B	C	C	GH	CD	CD	EF	AB	AB	BCD	A
Feb	Nov	Jan	Feb	Sep	Sep	Dec	Nov	Oct	Oct	Apr
B	C	C	GH	DE	D	EF	B	AB	CD	A
Nov	Sep	Nov	Jan	Aug	Jan	Jan	Jan	May	**Jun**	**Jun**
B	C	C	H	E	D	F	B	B	D	A

Table 9: Levels of significance for physical parameters measured at Clifton Springs (Site 13), derived by ANOVA, indicating temporal scales of variability. Significant effects ($p < 0.05$) shown in bold.

Type	NFR	Secchi	PAR	Temp	Salin
Year	**0.02**	0.15	**< 0.01**	**0.01**	**< 0.01**
Month	0.36	0.06	0.50	**< 0.01**	**< 0.01**
Year*Month	0.62	0.25	**0.01**	0.14	**< 0.01**

Table 10: Years which have the greatest mean value of each of the physical parameters measured at Clifton Springs (Site 13) are at the top of each column and are arranged in descending order to the least mean value for each of the parameters at the bottom of the column. The significance of the difference between the hierarchical annual mean values within each column is indicated by the capital letter under each year. Years with the same capital letters within each column have means which are not significantly different. The *R.* cf. *chunii* bloom years of 1993 and 1994 are shown in bold.

NFR	Secchi	PAR	Temp	Salin
1990	**1994**	1995	**1993**	1991
A	**A**	A	**A**	A
1991	1995	1992	1991	1990
AB	A	B	B	B
1995	1992	**1994**	1992	1992
B	A	**B**	B	B
1993	**1993**	1991	1995	1995
B	**A**	B	B	C
1994		**1993**	1990	**1994**
B		**B**	B	**D**
1992		1990	**1994**	**1993**
B		C	**B**	**E**

Table 11: Months which have the greatest mean value of each of the physical parameters measured at Clifton Springs (Site 13) are at the top of each column and are arranged in descending order to the least mean value for each of the parameters at the bottom of the column. The significance of the difference between the hierarchical monthly mean values within each column is indicated by the capital letter under each month. Months with the same capital letters within each column have means which are not significantly different. June (Jun) and July (Jul) are shown in bold as these are the two months preceding August, which appears to be the favoured month for bloom initiation when it occurs.

NFR	Secchi	PAR	Temp	Salin
Sep	May	May	Feb	May
A	A	A	A	A
Jul	**Jul**	Nov	Jan	Apr
AB	**A**	A	B	B
Dec	Apr	**Jun**	Mar	Mar
AB	A	**A**	BC	C
Aug	Mar	Sep	Dec	**Jun**
AB	AB	A	C	**C**
Feb	Dec	Mar	Nov	**Jul**
AB	AB	A	D	**D**
Jan	Feb	Jan	Apr	Feb
AB	AB	A	D	D
Jun	Jan	**Jul**	Oct	Aug
AB	ABC	**A**	E	D
Nov	Oct	Oct	May	Sep
AB	ABC	A	E	E
Oct	Nov	Feb	Sep	Jan
AB	ABC	A	F	EF
Apr	Aug	Dec	**Jun**	Oct
AB	ABC	A	**F**	FG
May	**Jun**	Aug	**Jul**	Nov
AB	**BC**	A	**G**	G
Mar	Sep	Apr	Aug	Dec
B	C	A	G	G

Table 12: Levels of significance for phytoplankton pigment parameters at Clifton Springs (Site 13), derived by ANOVA, indicating temporal scales of variability. Significant effects ($p < 0.05$) shown in bold.

Type	ChlA	ChlB	ChlC	Carot	Phaeo	LivA
Year	**0.04**	**0.02**	**< 0.01**	**0.03**	**< 0.01**	0.06
Month	**< 0.01**	**< 0.01**	**0.02**	0.37	0.81	**< 0.01**
Year*Month	**< 0.01**	**0.04**	**< 0.01**	**< 0.01**	**0.02**	**< 0.01**

Table 13: Years which have the greatest mean value of each of the plankton pigments measured at Clifton Springs (Site 13) are at the top of each column and are arranged in descending order to the least mean value for each of the parameters at the bottom of the column. The significance of the difference between the hierarchical annual mean values within each column is indicated by the capital letter under each year. Years with the same capital letters within each column have means which are not significantly different. The *R.* cf. *chunii* bloom years of 1993 and 1994 are shown in bold.

ChlA	ChlB	ChlC	Carot	Phaeo	LivA
1995	1992	1990	1995	1992	1995
A	A	A	A	A	A
1992	**1993**	1995	**1993**	**1993**	**1993**
AB	**A**	A	**AB**	**A**	**AB**
1993	1995	1992	1992	1991	1992
AB	A	AB	AB	B	AB
1991	1990	1991	1990	1995	1991
AB	AB	ABC	AB	B	AB
1990	1991	**1993**	1991	**1994**	1990
AB	BC	**BC**	B	**B**	B
1994	**1994**	**1994**	**1994**	1990	**1994**
B	**C**	**C**	**B**	B	**B**

Table 14: Months which have the greatest mean value of each of the plankton pigments measured at Clifton Springs (Site 13) are at the top of each column and are arranged in descending order to the least mean value for each of the parameters at the bottom of the column. The significance of the difference between the hierarchical monthly mean values within each column is indicated by the capital letter under each month. Months with the same capital letters within each column have means which are not significantly different. June (Jun) and July (Jul) are shown in bold as these are the two months preceding August, which appears to be the favoured month for bloom initiation when it occurs.

ChlA	ChlB	ChlC	Carot	Phaeo	LivA
Jul	**Jun**	**Jul**	**Jul**	May	**Jul**
A	**A**	**A**	**A**	A	**A**
Aug	**Jul**	Aug	Dec	**Jul**	Aug
B	**A**	AB	AB	**A**	AB
Dec	Apr	Feb	Aug	Dec	Dec
BC	AB	ABC	AB	A	BC
Jun	May	**Jun**	**Jun**	Jan	**Jun**
BC	ABC	**ABC**	**AB**	A	**BC**
May	Sep	Dec	May	Aug	May
BC	ABC	BC	AB	A	BC
Oct	Dec	May	Oct	Oct	Oct
BC	ABC	BC	AB	A	BC
Mar	Jan	Sep	Feb	Sep	Mar
BC	ABC	BC	AB	A	BC
Sep	Mar	Apr	Apr	**Jun**	Apr
BC	ABC	C	AB	**A**	BC
Apr	Oct	Jan	Mar	Mar	Sep
BC	BC	C	AB	A	BC
Jan	Aug	Mar	Jan	Nov	Jan
BC	BC	C	AB	A	BC
Feb	Feb	Oct	Sep	Apr	Feb
C	C	C	B	A	BC
Nov	Nov	Nov	Nov	Feb	Nov
C	C	C	B	A	C

Table 15: Levels of significance for certain meteorological parameters, derived by ANOVA, indicating temporal scales of variability. Significant effects ($p < 0.05$) shown in bold.

Type	SWS	TDR	TCA	DBT	WBT	DPT	Rain
Year	0.12	**0.04**	0.12	**< 0.01**	**< 0.01**	**< 0.01**	0.25
Week	0.41	**< 0.01**	0.99	**< 0.01**	**< 0.01**	**< 0.01**	0.34
Year*Week	**< 0.01**	0.29	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**

Table 16: Mean weekly values of meteorological parameters for each of the six weeks prior to 4th August are arranged in descending order of magnitude within each column. Week 1 signifies the week immediately prior to 4th August, the approximate date of bloom initiation. The significance of the difference between the hierarchical weekly mean values within each column is indicated by the capital letter under each week. Weeks with the same capital letters within each column have means which are not significantly different.

SWS	TDR	TCA	DBT	WBT	DPT	Rain
1	1	3	5	5	5	3
A	A	A	A	A	A	A
2	2	4	1	1	6	1
A	AB	A	AB	B	A	AB
6	3	1	4	6	3	5
A	BC	A	B	B	A	AB
5	4	6	3	3	1	6
A	C	A	BC	B	A	AB
4	6	2	6	4	4	4
A	C	A	BC	B	A	AB
3	5	5	2	2	2	2
A	C	A	C	C	B	B

Table 17: Top ten species by mean concentration (cells L^{-1}) at Clifton Springs over a 6 week period beginning (a) six weeks and (b) twelve weeks weeks prior to August for the years 1988-95. Species shown in bold are common with the bloom years 1993 and 1994.

(a) Mid-June to July (6 weeks)

1988		1989		1990		1991		1992		1993		1994		1995	
species	conc	species	conc	species	conc	species	conc	species	conc	species	conc	species	conc	species	conc
2176	505550	2165	271000	2174	438000	1117	560000	**4009**	**218000**	**7026**	**1370000**	**4009**	**522000**	7026	1176000
4013	**309925**	4003	140000	2076	352000	**4009**	**411000**	**4013**	**160000**	**4009**	**540000**	**4013**	**480000**	1030	456000
6034	**61450**	2076	130000	1117	124800	**2203**	**197800**	**1049**	**109000**	**4013**	**517800**	**4007**	**248000**	2071	364000
1115	48730	2174	117000	4011	116800	**7026**	**196600**	**7026**	**86000**	**4007**	**280000**	**7026**	**192000**	6034	112000
8003	39500	**7026**	**54800**	**7026**	**86000**	1115	149000	**6034**	**63000**	**1049**	**253000**	6009	184000	7008	104000
7026	**26920**	**4009**	**47000**	2069	78000	**6034**	**125800**	1115	47000	**2203**	**120000**	**1049**	**136000**	4009	88000
1176	19250	**4013**	**38800**	1115	70200	2174	78800	2174	47000	1116	70000	**2140**	**43000**	2174	80000
1116	11550	1117	31000	2188	70000	6010	55000	1116	16000	**6034**	**70000**	1115	40000	1115	72000
2092	10615	**6034**	**31000**	**6034**	**55000**	1046	47000	2092	16000	**2140**	**47000**	**2203**	**17000**	7004	56000
2174	10615	1115	30800	**4009**	**31600**	1116	47000	2107	16000	1042	23000	**6034**	**16000**	1049	40000

(b) May to mid-June (6 weeks)

1988		1989		1990		1991		1992		1993		1994		1995	
species	conc	species	conc	species	conc	species	conc	species	conc	species	conc	species	conc	species	conc
7026	**145100**	**7026**	**194000**	2174	327800	6034	109000	4009	498000	**7026**	**558000**	**4013**	**112000**	**7026**	1056000
2188	65280	6034	147000	6034	244000	**1115**	**101800**	**7026**	**346000**	4009	410000	**7026**	**104000**	6034	208000
2016	55280	6009	127800	**7026**	**214800**	4009	101600	6010	86000	2174	216000	**9029**	**96000**	7004	144000
2076	49920	6010	69800	1117	167800	2019	94000	6034	86000	**1115**	**86000**	2165	88000	7008	104000
1025	38820	1061	62000	4009	117000	6002	94000	**4013**	**48000**	**4013**	**61800**	**1115**	**40000**	4009	96000
4029	30720	4009	62000	6010	116000	2174	93600	1040	47000	1004	46400	4010	40000	7018	88000
1115	**20740**	1116	39000	**1115**	**109400**	1117	86000	2174	47000	6024	39000	9023	32000	1030	56000
2165	20740	2107	31000	2165	94000	6009	85800	**1115**	**32000**	6034	39000	2191	24000	1049	40000
1030	15540	**1115**	**30800**	4007	86000	6010	79000	6009	32000	**9029**	**30800**	3007	16000	2174	40000
2107	7700	6017	23800	6002	70800	**7026**	**79000**	6036	16000	2076	16000	6010	16000	1115	32000

Class codes: 1000-1999 Dinophyceae; 2000-2999 Diatomophyceae; 3000-3999 Raphidophyceae; 4000-4999 Cryptophyceae; 5000-5999 Euglenophyceae; 6000-6999 Prymnesiophyceae; 7000-7999 Prasinophyceae; 8000-8999: Dictyochophyceae; 9000+ others. Planktonic species too small for valid routine identification (eg. picoplankters) have not been considered.

Table 18: The eight most abundant species or taxa, and their cell concentration (cells L^{-1}) , at Clifton Springs in July 1993 and 1994, and at Point Richards in July 1997.

13 July 1993		6 & 21 July 1994		17 & 23 July 1997	
Species	Conc.	Species	Conc. *	Species	Conc. *
Pyramimonas spp.	810,000	***Teleaulax axuta***	220,000	*Chrysochromulina spp.*	220,000
Plagioselmis prolonga	260,000	*Hemiselmis vivescens*	1146,000	***Pyramimonas spp.***	118,000
Katodinium rotundatum	93,000	***Plagioselmis prolonga***	92,000	***Plagioselmis prolonga***	102,000
Teleaulax acuta	78,000	*Emiliania huxleyi*	80,000	*Rhomdomoas salina*	92,000
Chrysochromulina spp.	70,000	***Katodinium rotundatum***	68,000	***Katodinium rotundatum***	89,000
Gephyrocarpsa oceanica	16,000	***Pyramimonas spp.***	36,000	*Gymnodinium spp.*	72,000
Scrippsiella spp.	7,800	*Gymnodinium spp.*	16,000	*Thalassiothrix spp.*	70,000
Caerataulina pelagica	7,800	*Pteridomonas danica*	4,000	***Teleaulax axuta***	44,000

* mean cell concentration for two specified dates
Species in **bold** are common to all three years

Table 19: Periods between 1968 and 1995 in which the salinity of Port Phillip Bay fell below 34.5, and the timing of *R.* cf. *chunii* blooms at the seven key sites.

Year	Period of year < 34.5	July and August < 34.5	*R.* cf. *chunii* blooms in Port Phillip Bay
1968	None	No	
1969	None	No	
1970	None	No	
1971-74	No data	-	
1975	Oct-Dec (no data before Oct)	Probably*	Probable**
1976	May-June	No	
1977-79	No data	-	
1980	Sep-Dec	No	
1981	Feb-June, Oct-Dec	No	
1982	Feb-Apr	No	
1983	None	No	
1984	None	No	
1985	Oct-Dec	No	
1986	Jan-Feb	No	
1987	Aug	Aug only	Aug-Oct
1988	None	No	
1989	No data	-	
1990	Oct	No	
1991	None	No	
1992	Oct-Dec	No	
1993	Jan-Dec	Yes	Aug-Sep
1994	Jan-Nov	Yes	Aug-Sep
1995	Aug-Dec	Aug only	
1996	Jan, Jul-Dec	Yes	
1997	None	No	Aug-Sep

* Salinity in October-December 1975 was less than 34.0, implying significant freshwater input to the Bay in earlier months.

** The bloom species causing bitter taste in shellfish in 1975 was not identified.

FIGURES

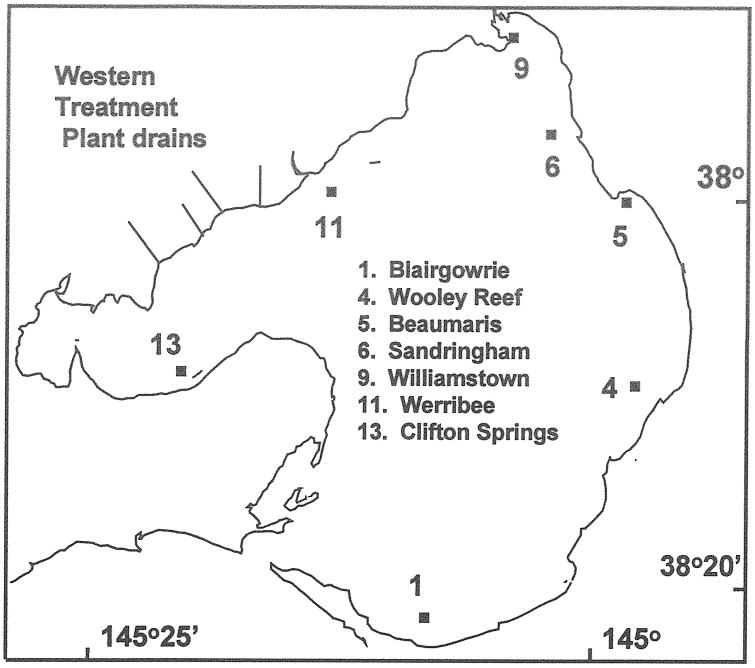


Figure 1a: The seven key sites sampled in Port Phillip Bay for phytoplankton from 1987 - 1995.

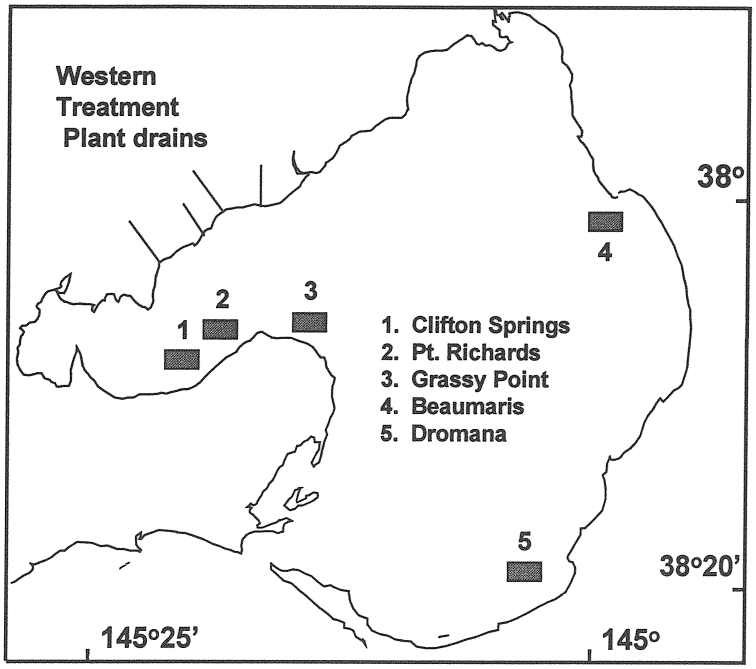


Figure 1b: The five mussel aquaculture zones in Port Phillip Bay.

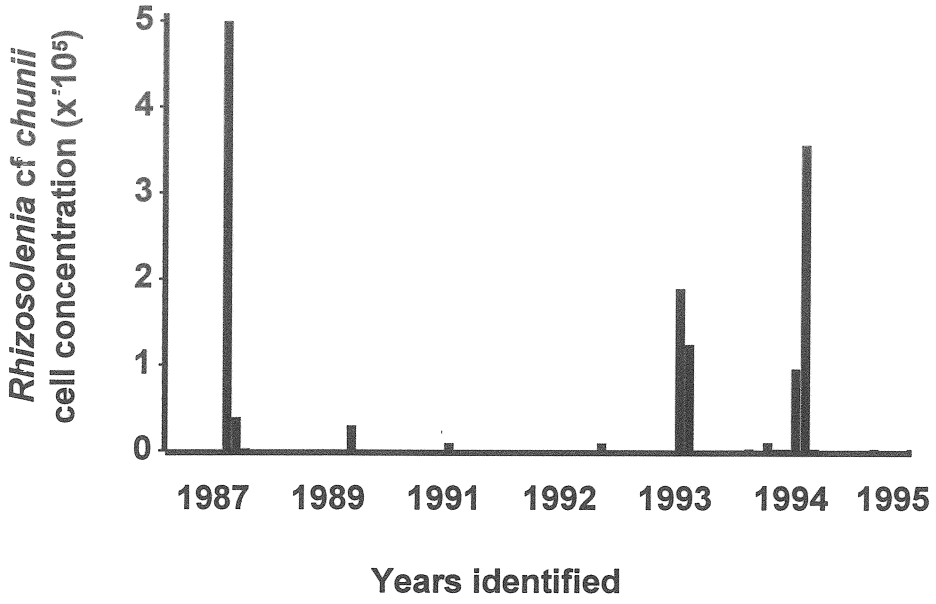


Figure 2: Monthly mean concentrations of *R.* cf. *chunii* (data from all seven key sites polled) for those years between 1987-1995 when it was observed in countable numbers in the Bay.

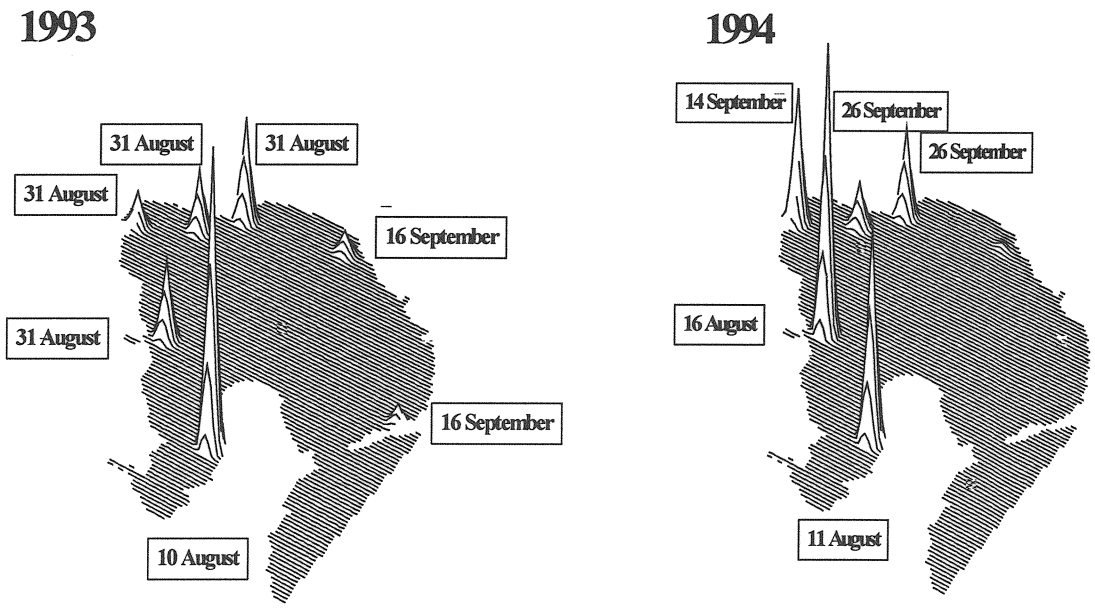


Figure 3: Cell counts of *R.* cf. *chunii* at the seven key sampling sites during the 1993 and 1994 bloom periods (August-September), and the date of first occurrence of *R.* cf. *chunii* in cell concentrations greater than 50,000 cells L^{-1} at each site.

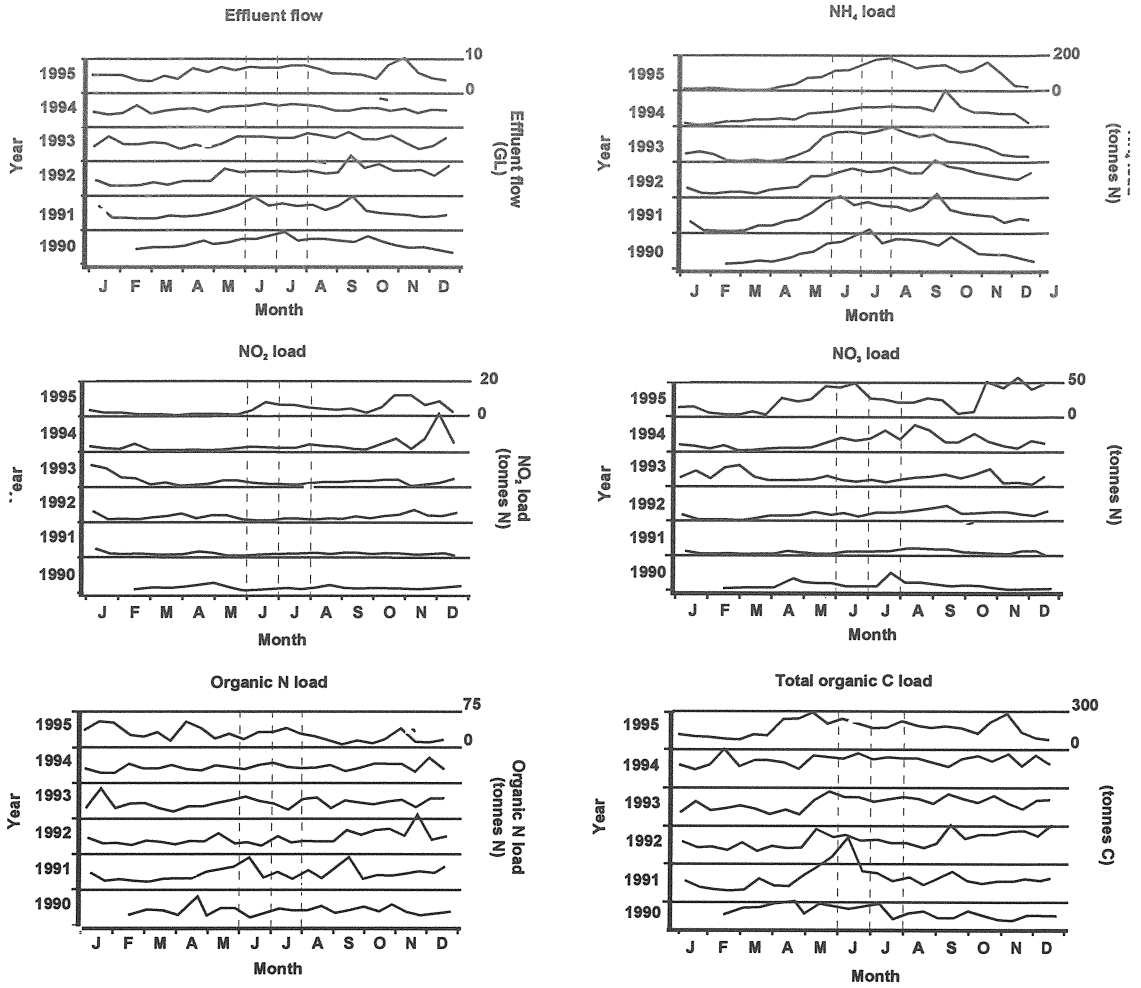


Figure 4a: Fortnightly effluent flow and NH_4, NO_2, NO_3, organic N and total organic C loads from the Western Treatment Plant during 1990-95. Vertical dashed lines mark the start of the three winter months.

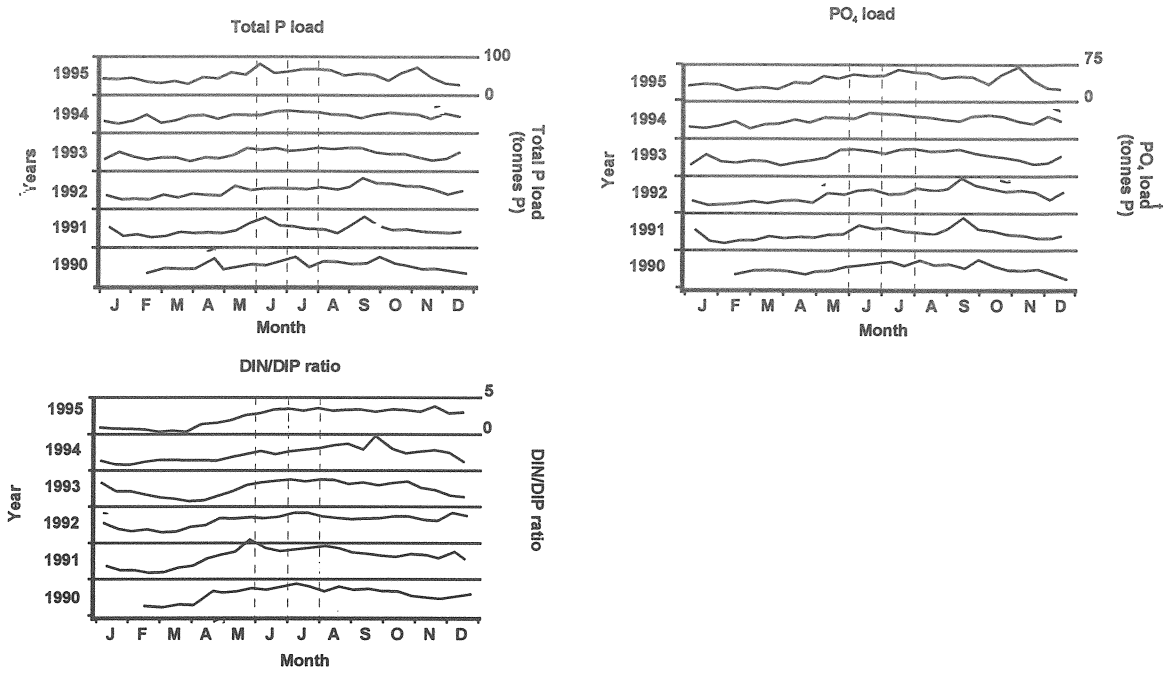


Figure 4b: Fortnightly total P and PO_4 loads, and DIN:DIP ratio, in the effluent from the Western Treatment Plant during 1990-95. Vertical dashed lines mark the start of the three winter months.

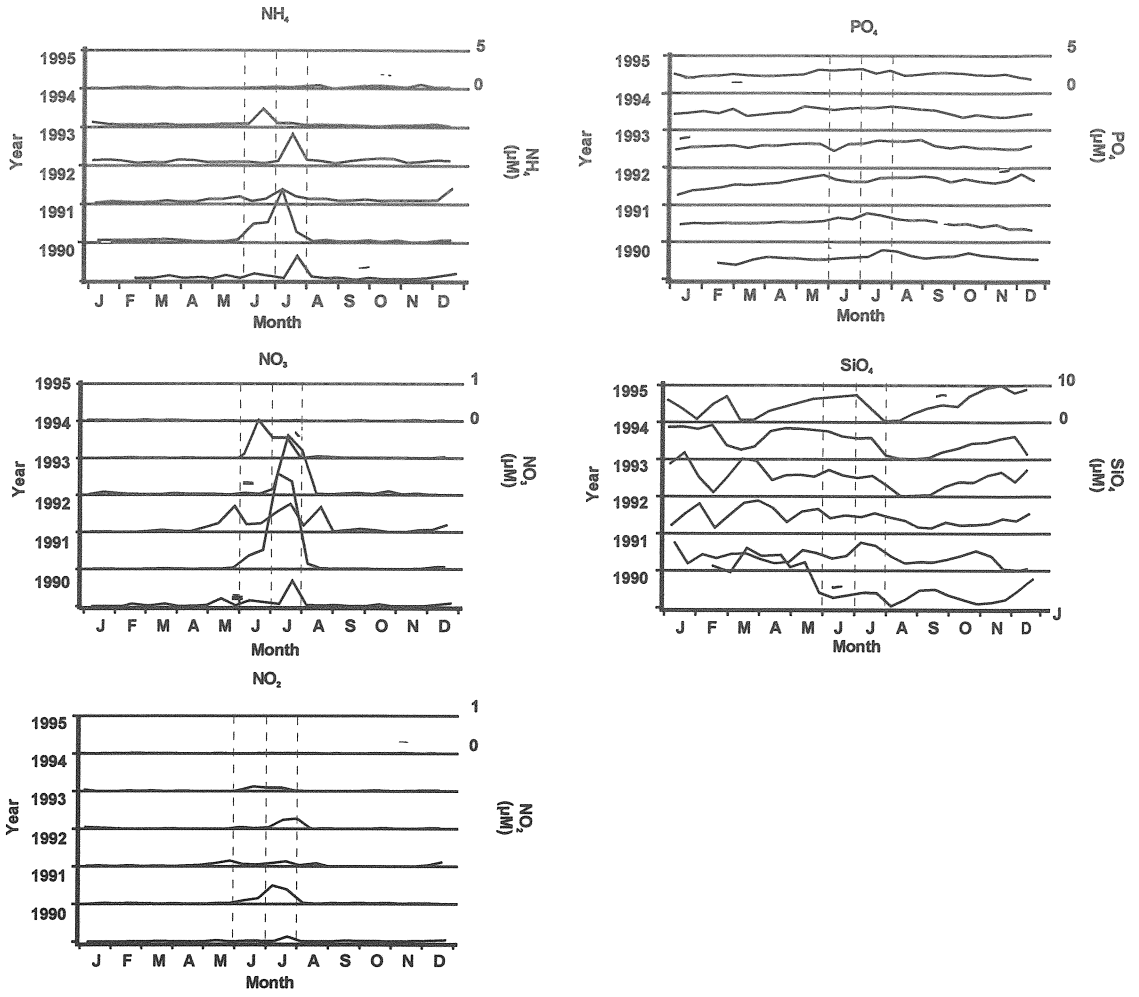


Figure 5a: Water column concentrations (μm) of the inorganic nutrients NH_4, NO_3, NO_2, PO_4 and SiO_4 measured fortnightly at Clifton Springs (site 13) from 1990-95. Vertical dashed lines mark the start of the three winter months.

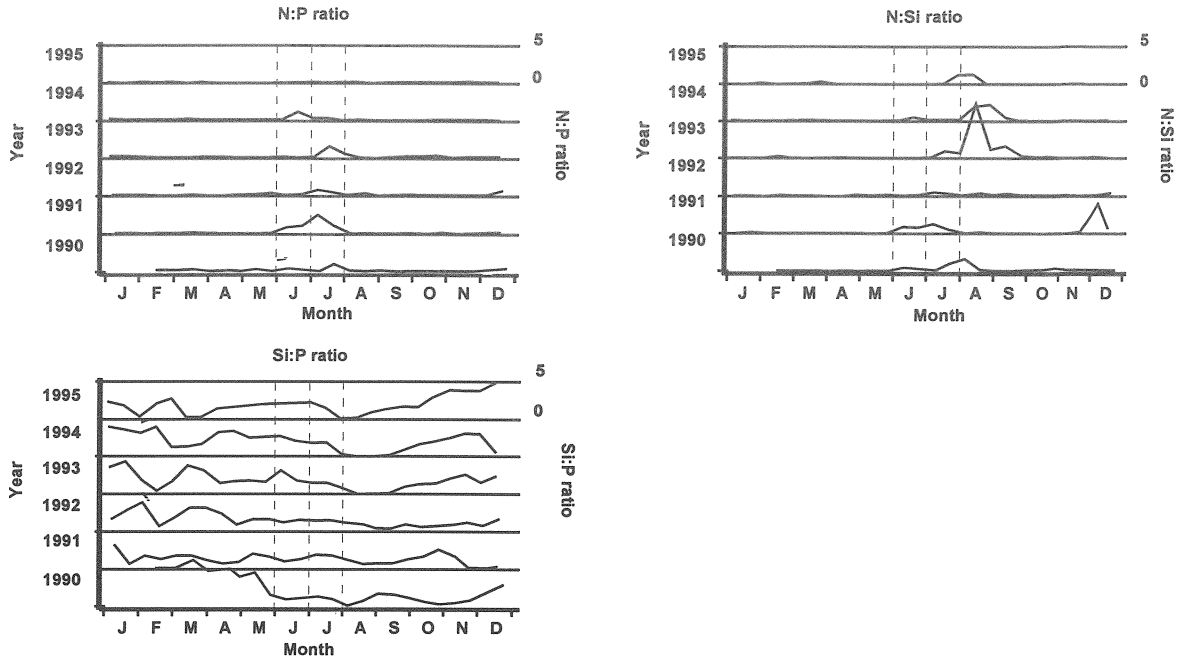


Figure 5b: Ratios of ambient inorganic nutrients at Clifton Springs (site 13) from 1990-95. Vertical dashed lines mark the start of the three winter months.

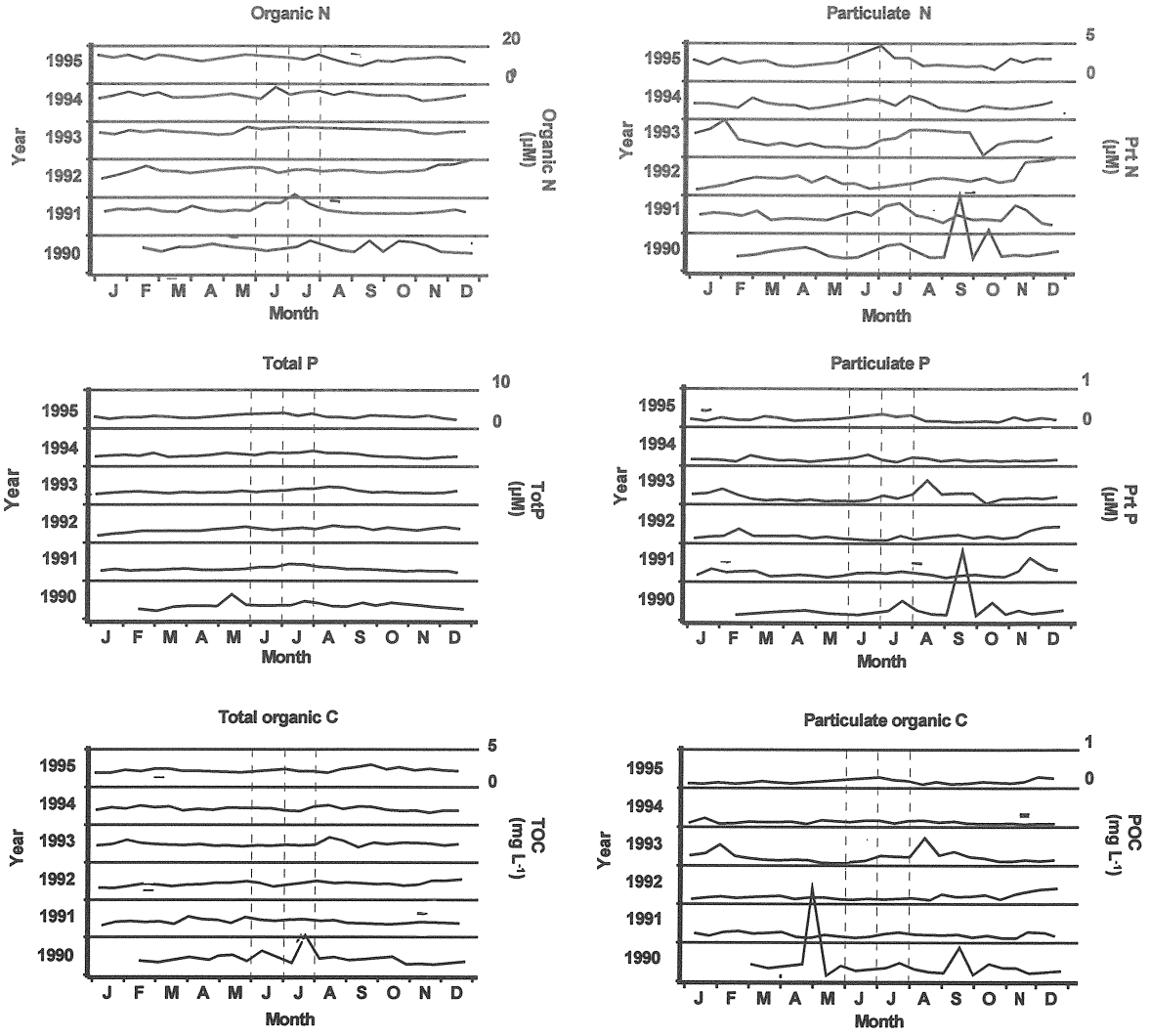


Figure 5c: Water column concentrations of organic and particulate N, total and particulate P and total and particulate organic C measured fortnightly at Clifton Springs (site 13) from 1990-95. Vertical dashed lines mark the start of the three winter months.

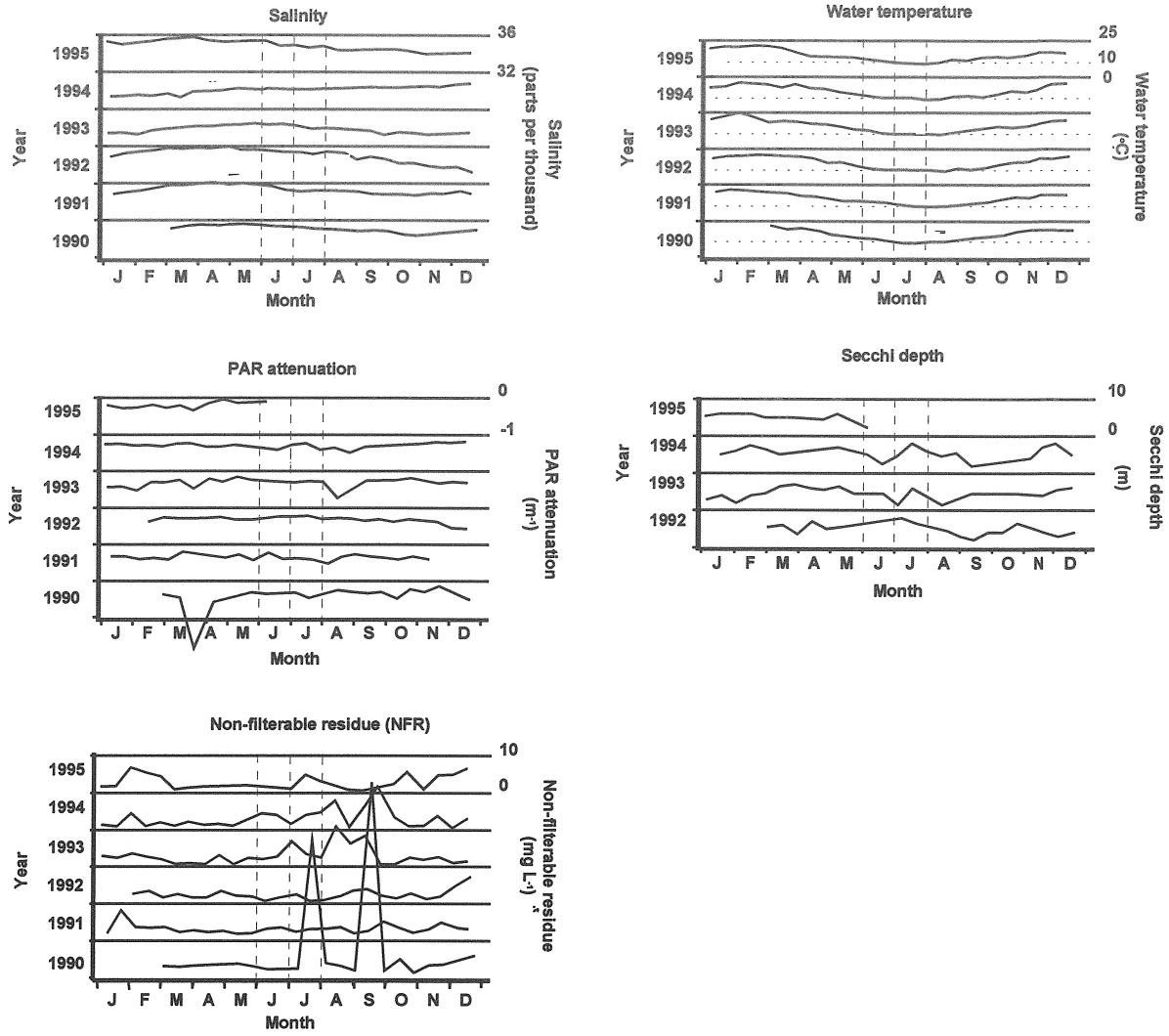


Figure 5d: Measurements of physical properties of the water column taken fortnightly at Clifton Springs (site 13) from 1990-95. Secchi depth data for 1990 and 1991 was unavailable. Vertical dashed lines mark the start of the three winter months.

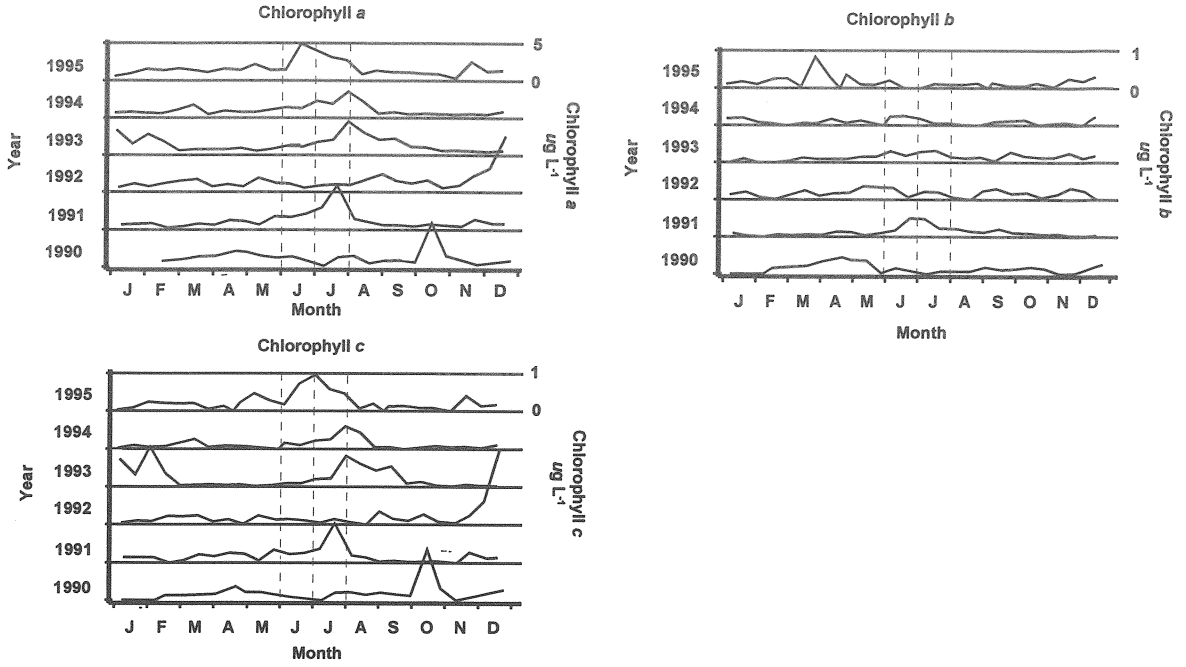


Figure 6a: Water column concentrations of chlorophylls *a*, *b* and *c* at Clifton Springs (site 13) from 1990-95. Vertical dashed lines mark the start of the three winter months.

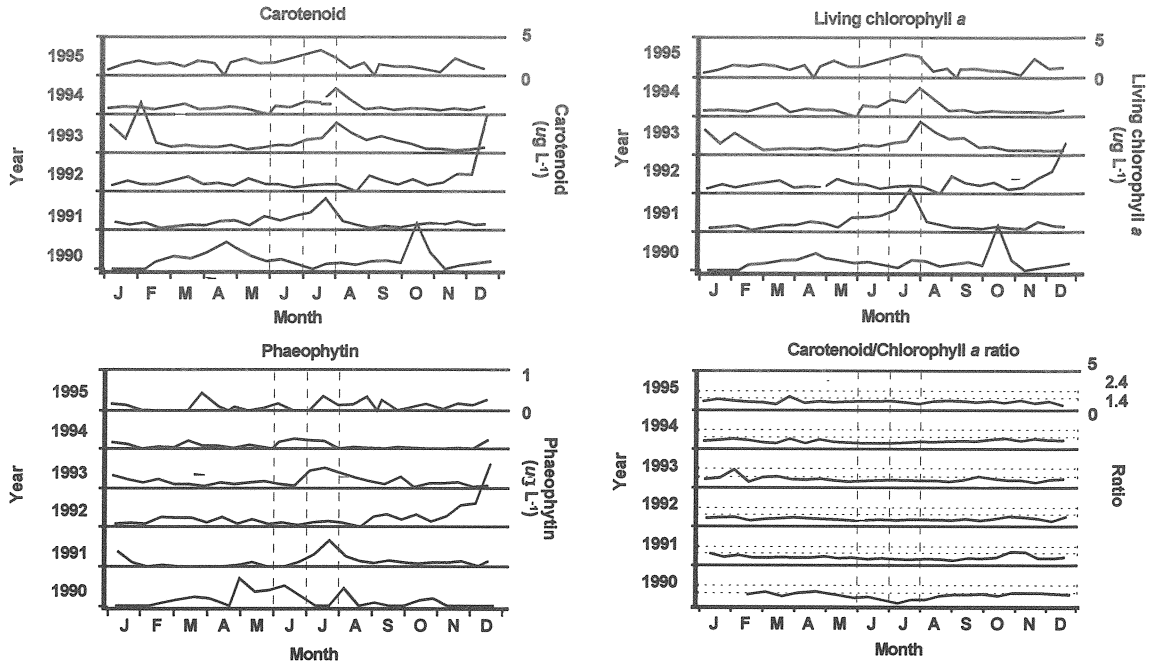


Figure 6b: Water column concentrations of carotenoid, living chlorophyll *a*, phaeophytin and the carotenoid:chlorophyll *a* ratio, at Clifton Springs (site 13) from 1990-95. The upper and lower dotted lines in the figure for carotenoid:chlorophyll *a* ratio indicate ratio values of 2.4 and 1.4 respectively. Vertical dashed lines mark the start of the three winter months.

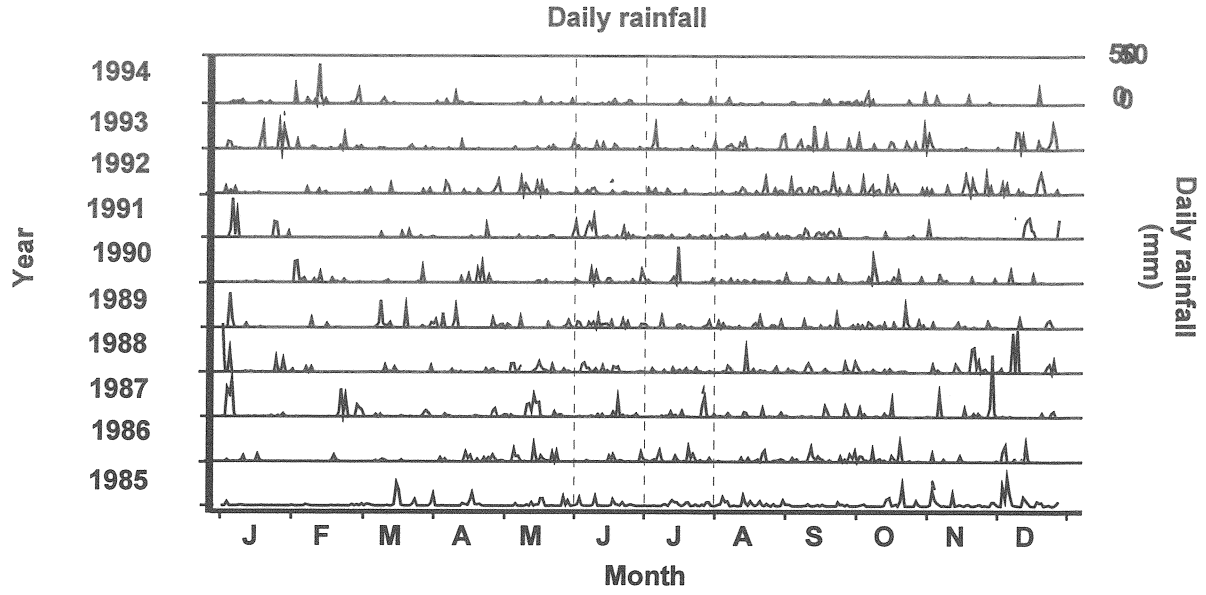


Figure 7: Mean of the daily rainfall measured at Portarlington, Geelong and Werribee gauging stations from 1985 to 1994. Data for 1995 was unavailable at the time of analysis. Vertical lines mark the start of the three winter months.

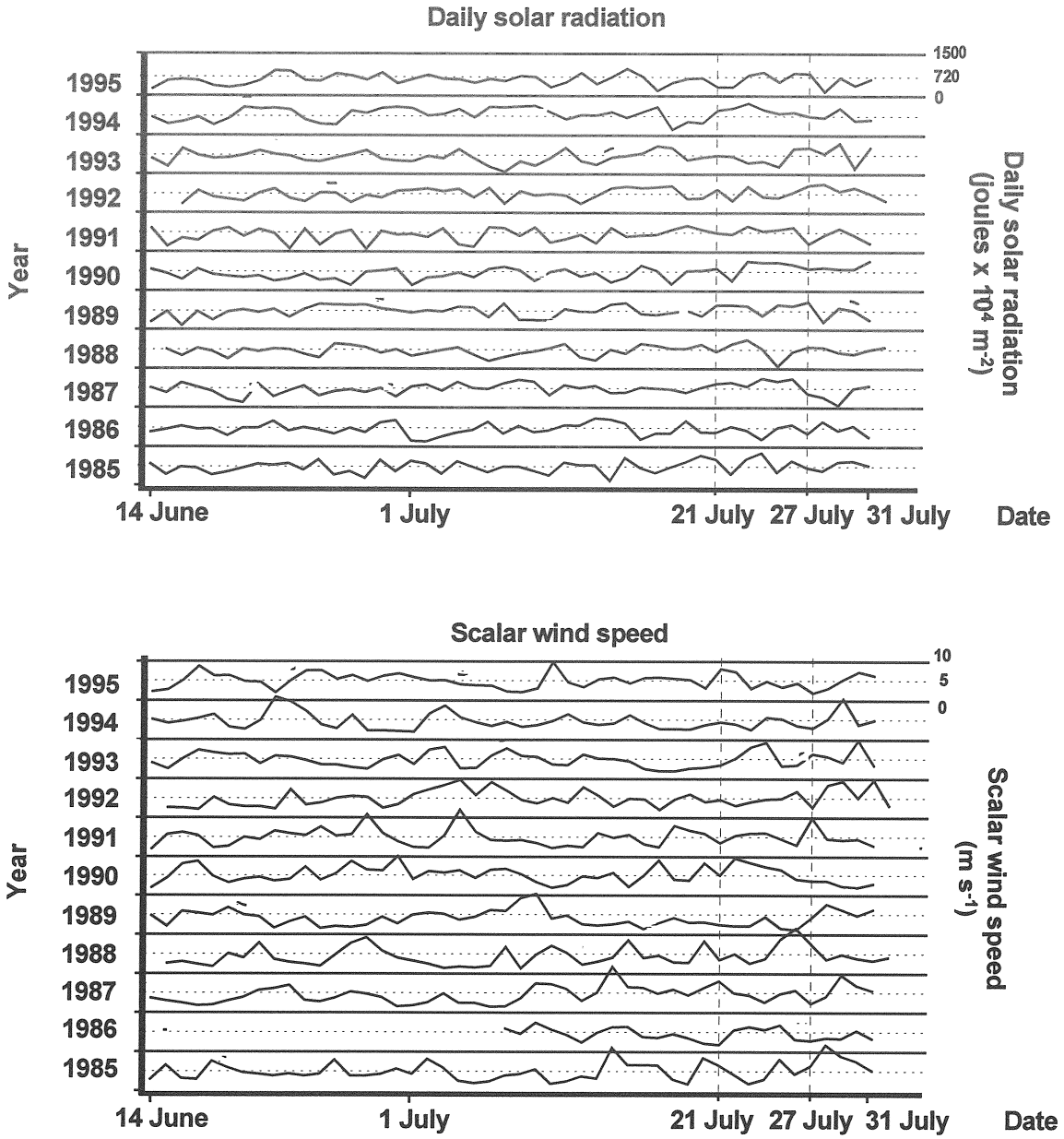


Figure 8: Daily solar radiation (top) and daily mean scalar wind speed (bottom) for the 6 weeks preceding August from 1985 to 1995. Vertical dashed lines indicate dates 8 and 14 days before the date in early August of 1993 and 1994 when increased concentrations of *R.* cf. *chunii* were first observed at Clifton Springs (Site 13).

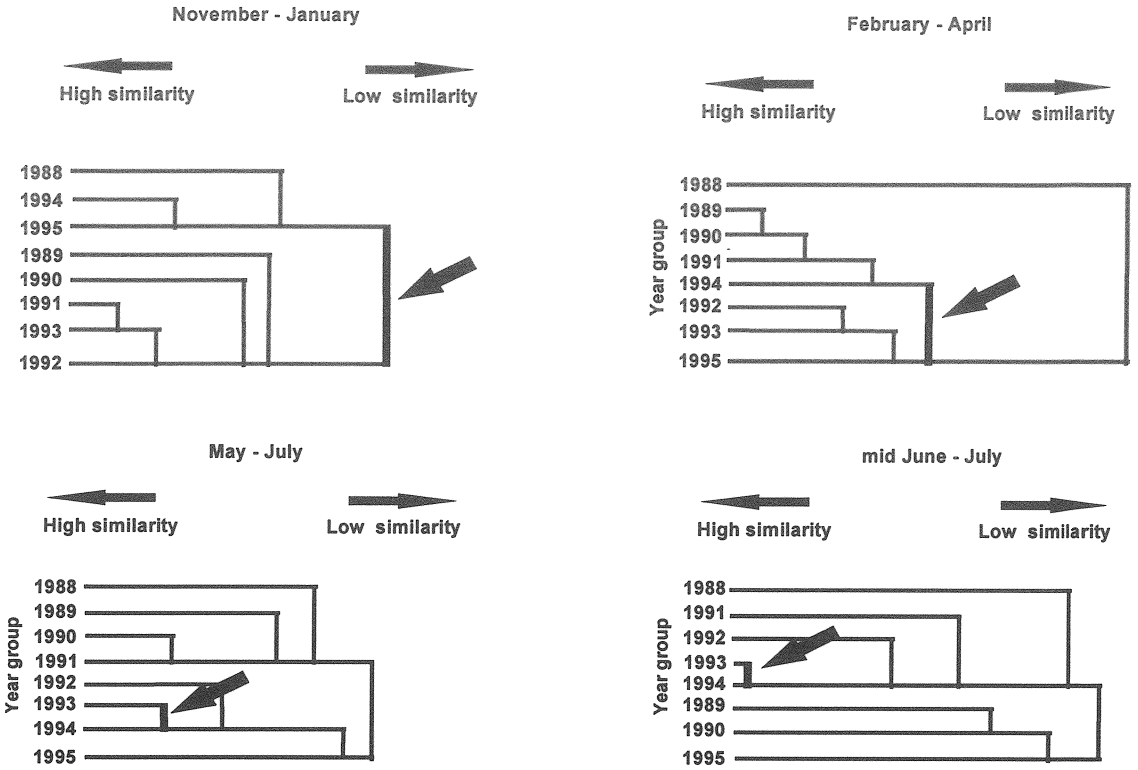


Figure 9: Dendrograms derived from pattern analysis of phytoplankton composition and abundance data for Clifton Springs during four time periods in the years 1988-1995. Arrows highlight the level of similarity between planktonic communities present in 1993 and 1994, the two years in which *R.* cf. *chunii* bloomed.

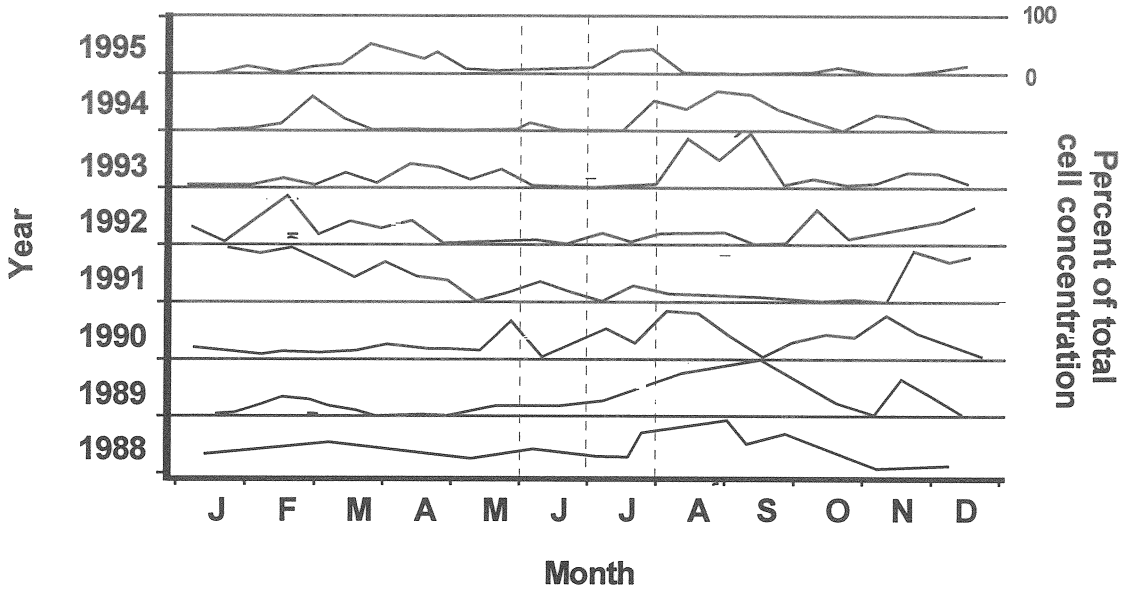


Figure 10: Monthly variation in the proportion of diatoms present in the phytoplankton at Clifton Springs from 1988 to 1995. Vertical dashed lines mark the beginning of the three winter months.

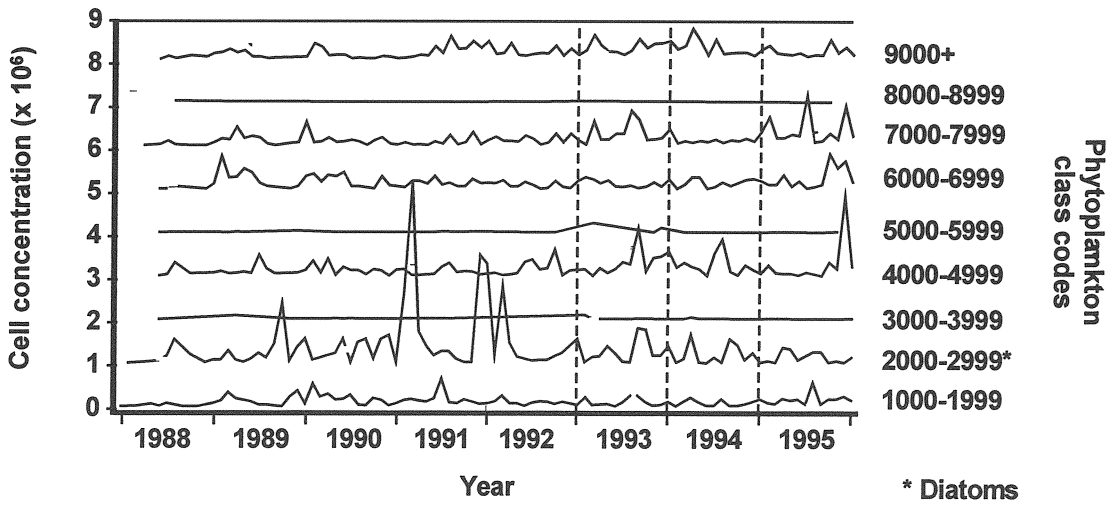


Figure 11: Phytoplankton cell concentrations by class at Clifton Springs from 1988 - 1995. The vertical dashed reference lines delineate 1993 and 1994. Classes and their respective codes are provided in Table 17. The left-hand scale is common to all classes but the baseline is sequentially offset by 1 x 10^6 units per increasing class code.

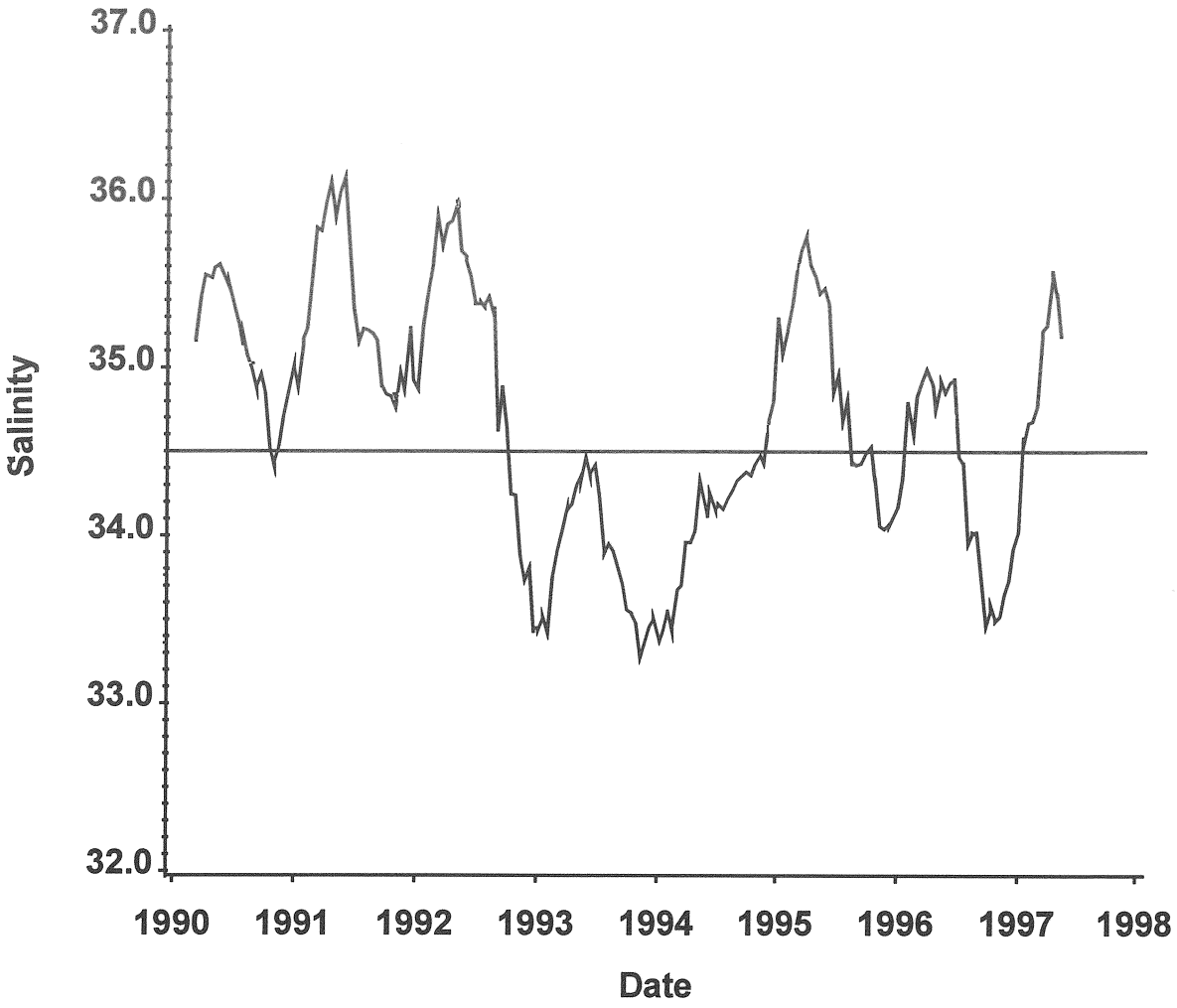


Figure 12: Salinity in bottom waters at Clifton Springs from March 1990 to May 1997.

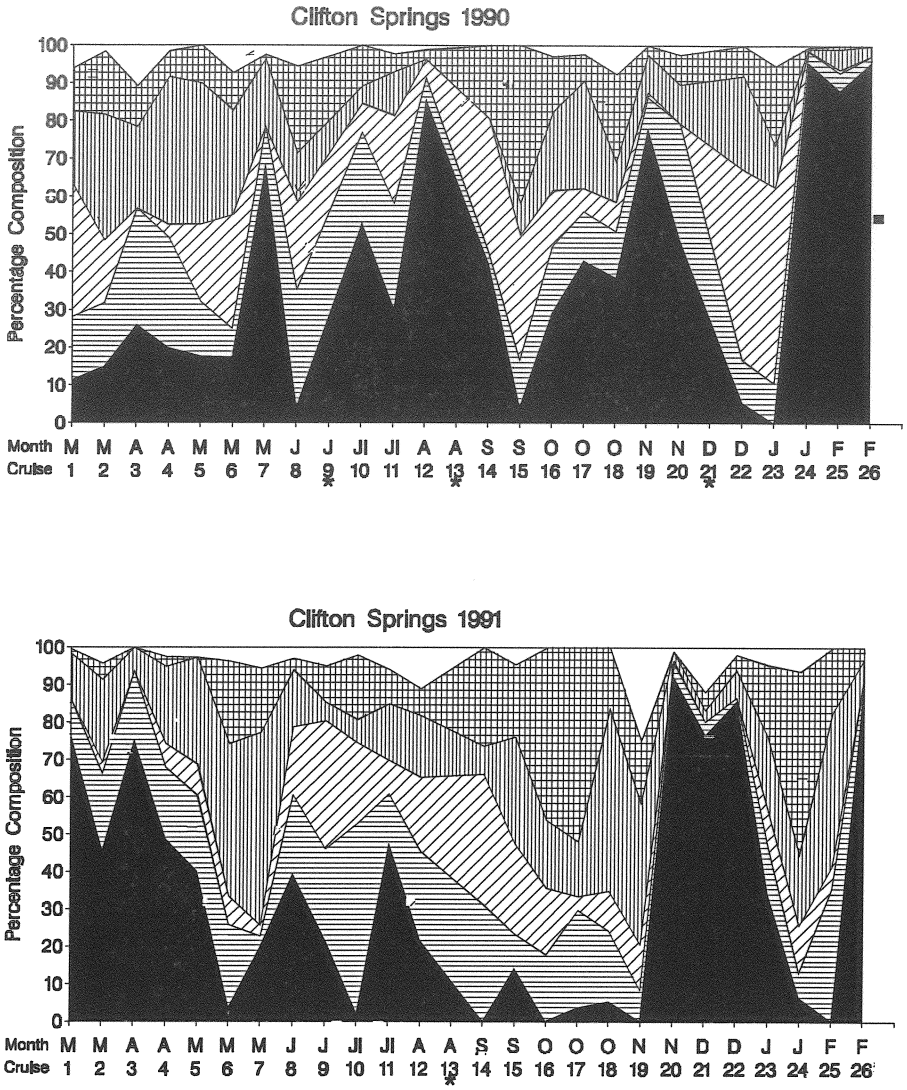
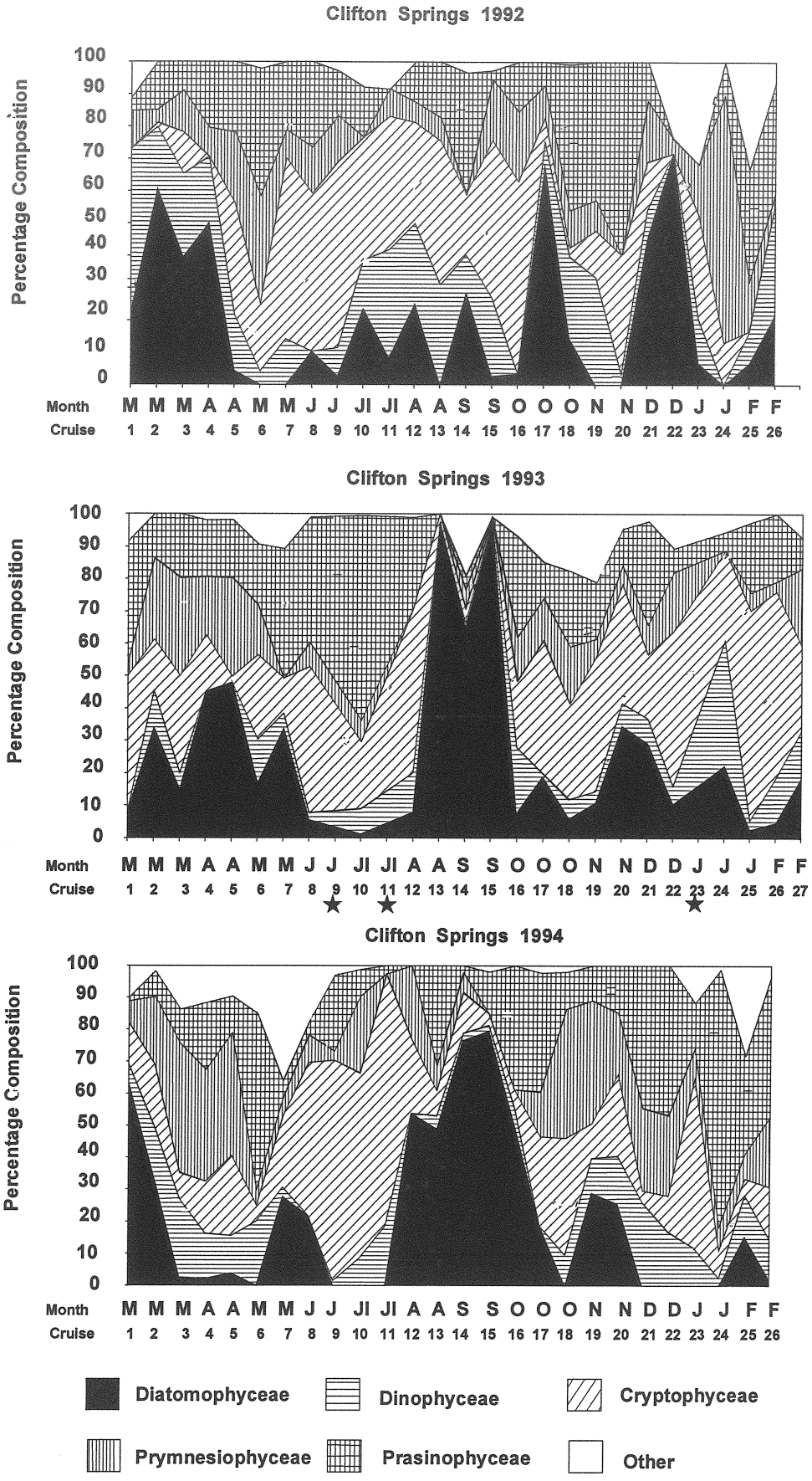


Figure 13. The variation in the relative abundance of the five main classes of phytoplankton, and 'other' classes combined, by month and cruise for each year at Clifton Springs. * indicates missing sample. [After Arnott et al., 1997; note nominal year runs from March to following February].

Continued....


Clifton Springs 1992
Percentage Composition
100
90
80
70
60
50
40
30
20
10
0
Month M M M A A M M J J Jl Jl A A S S O O O N N D D J J F F
Cruise 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
Clifton Springs 1993
Percentage Composition
100
90
80
70
60
50
40
30
20
10
0
Month M M M A A M M J J Jl Jl A A S S O O O N N D D J J J F F
Cruise 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
Clifton Springs 1994
Percentage Composition
100
90
80
70
60
50
40
30
20
10
0
Month M M M A A M M J J Jl Jl A A S S O O O N N D D J J F F
Cruise 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
Diatomophyceae
Dinophyceae
Cryptophyceae
Prymnesiophyceae
Prasinophyceae
Other

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