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Phytoplankton in Port Phillip Bay: Spatial and seasonal trends in biomass and primary productivity.

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EXECUTIVE SUMMARY

Phytoplankton in three size fractions - pico (0.2 to 2 microns), nano (2to20 microns) and micro (> 20 microns) were collected from nine stations in Port Phillip Bay from August,1993, to April,1995. Collections were made at surface, mid-depth and bottom.

The chlorophyll *a* content of each sample was determined by filtration, extraction and either spectrophotometry or fluorimetry or both.

The productivity of each sample was measured by incubation with $H^{14}CO_3$ and subsequent scintillation counting.

Ancillary collections were also made to examine small-scale temporal and spatial variations in biomass and productivity.

Stations near the coast showed consistently higher biomass than those in the centre and south of the Bay. The latter displayed a clear seasonal signal with highest values in autumn whilst the former showed more irregular temporal variation. The three size fractions contributed equally to the mean concentrations found (0.47,0.51 and 0.62 mg/m^3). However, this concealed a much greater range of values (0 to 8.mg/m^3) in the micro fraction with a median value of only 0.2mg/m^3.

Depth differences were observed with coastal stations generally showing higher chlorophyll concentrations at the surface and central stations the converse.

Notwithstanding concentration differences, central stations contained more chlorophyll per unit area because of their greater depth.

For the total phytoplankton population, productivity reached a peak in the autumn of 1994 with a lesser rise through the autumn of 1995. This composite pattern, however, concealed marked differences with season and position between the three size fractions. These differences are described and interpreted in relation to driving forces including light.

Bay-wide productivity tended to a maximum in late summer/early autumn in both 1994 and 1995. There were no marked spatial differences but the three northern stations tended to slightly higher productivity overall. Total carbon fixation ranged from about 200mg/m^2 /day in winter to 400mg/m^2 /day or more in the other three seasons. It was estimated that Bay-wide annual carbon fixation amounts to 260,000 tonnes.

The authors conclude that the three size fractions present significantly different ecological patterns confounded by Bay-wide spatial effects.

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1. INTRODUCTION

This report presents the results of studies into the seasonal and spatial distribution of phytoplankton biomass and productivity in Port Phillip Bay, Victoria. There have been few previous studies of the phytoplankton of Port Phillip Bay. Of those investigations that have been carried out, many have been restricted spatially (e.g. Grant and Gayler 1982; Axelrad et al. 1979,1981) and the only reports of long-term trends concern studies of phytoplankton biomass rather than productivity (Longmore et al. 1990; Saunders and Goudey 1990). Whilst some surveys (Arnott 1990) have examined the species composition of the phytoplankton (concentrating on potentially toxic species) in selected areas of the Bay, there have been no systematic studies of the size composition of the phytoplankton and the contribution to biomass and productivity of the potentially important nano- and pico-plankton (Jiao and Wang 1994; Furnas and Mitchell 1987; Sellner 1983; Abreu 1994; Revelante and Gilmartin 1978). A more detailed appraisal of the literature pertaining to the phytoplankton ecology of Port Phillip Bay has been provided by Wood and Beardall (1992).

Although there is quite an extensive data base containing chlorophyll a concentrations in Port Phillip Bay between 1970 and 1990, these data do not address a number of important points such as descriptions of vertical distribution of chlorophyll a, changes in chlorophyll a at scales of 100s metres and kms, variations in phytoplankton biomass over scales of the order of days and the contributions of particular groups within the population to the total chlorophyll a concentration. In addition, the general lack of basic data on primary production rates makes it very difficult to assess the overall state of the phytoplankton component of the ecosystem. Data that would allow determination of the factors that limit growth and/or biomass are also lacking.

The present study set out therefore to address the following aims:

1. To describe the seasonal and spatial distribution of phytoplankton biomass in Port Phillip Bay;
2. To describe the seasonal and spatial distribution of phytoplankton productivity in the Bay;
3. To determine the contribution of different size fractions (micro-, nano- and pico-plankton) to standing stock and productivity of phytoplankton in the Bay;
4. To arrive at estimates for annual primary production by phytoplankton in the Bay.

2. METHODS

2.1. Sampling procedures

Ten stations were identified as providing adequate coverage of the Bay whilst still permitting sampling of all stations within a two day cruise. These stations are shown in Figure 1.

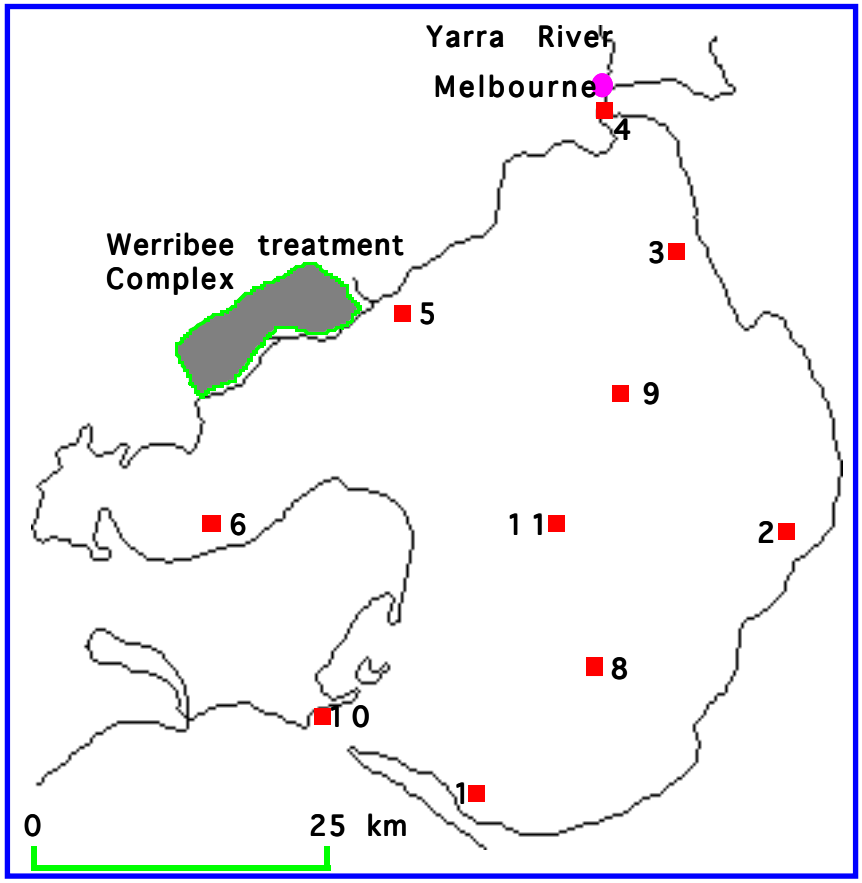


Figure 1: Stations sampled during the period August 1993-April 1995

Two different frequencies of sampling were used. In the first, samples were collected every 4 weeks from stations 1-11 (Figure 1). All stations were sampled between August 1993 and April 1995 except station 11 from which samples were collected only between January 1994 and April 1995, and station 10 which was discontinued early in the program. At each station, water samples were collected from surface, mid and bottom depths with a submersible pump. Water from surface, mid-depth and bottom was used to determine total chlorophyll *a* concentration and the chlorophyll *a* content for the micro- (> 20 μm), nano- (2-20 μm) and pico-plankton (0.2-2 μm) fractions. These are hereafter referred to as micro, nano and pico fractions. *In situ* profiles of fluorescence were also obtained at each station using a *Seatech* fluorometer.

Samples from mid water were incubated with $H^{14}CO_3$ to determine carbon fixation rates. Four or five stations were selected, every four weeks, for a full incubation of samples at 6 different light intensities for construction of P vs I curves. At the remaining stations, only determinations of light saturated rates of carbon fixation were made. All fixation rate incubations were made for the total population as well as for the micro, nano and pico fractions of the population. Carbon fixation rates were not determined for station 10.

In the second sampling program, designed to examine short-term variation in phytoplankton characteristics, samples were collected from a total of 7 or 8 stations per day. This routine was repeated every 3-4 days with a two day interval between cruises. A suite of 3 such cruises were completed on each of 3 occasions :- November-December 1993, May 1994 and September 1994. The sampling dates for particular stations are presented in Table 1.

Table 1: Sampling frequency and photosynthetic parameters measured during short-term variation studies

Date	Station									
	3	3a	3b	5	5a	8	8a	8b	11	11a
26/11/93	P vs I	P_{max}	P_{max}	P vs I	P_{max}	P vs I	P_{max}	P_{max}		
29/11/93	P vs I	P_{max}	P_{max}	P vs I		P vs I	P_{max}	P_{max}		
2/12/93	P vs I	P_{max}	P_{max}	P vs I		P vs I	P_{max}	P_{max}		
13/5/94				P vs I	P_{max}				P vs I	
16/5/94	P vs I	P_{max}	P_{max}	P vs I	P_{max}				P vs I	P_{max}
20/5/94	P vs I	P_{max}	P_{max}	P vs I	P_{max}				P vs I	P_{max}
21/9/94	P vs I	P_{max}	P_{max}	P vs I	P_{max}				P vs I	P_{max}
23/9/94	P vs I	P_{max}	P_{max}	P vs I	P_{max}				P vs I	P_{max}
26/9/94	P vs I	P_{max}	P_{max}	P vs I	P_{max}				P vs I	P_{max}

On any given day, samples were collected from surface, mid and bottom depths for determination of total chlorophyll *a* concentration and that due to the micro-, nano- and pico- fractions. A profile of *in situ* fluorescence was obtained with a SeaTech fluorometer. Samples were collected from mid water and incubated with $H^{14}CO_3$ for determination of carbon fixation rates. On any given day samples from a total of 3 stations were incubated at 6 light intensities for construction of P vs I curves. At the remaining stations, samples were only incubated at saturating light intensities for determination of light saturated rates of carbon fixation (Table 1).

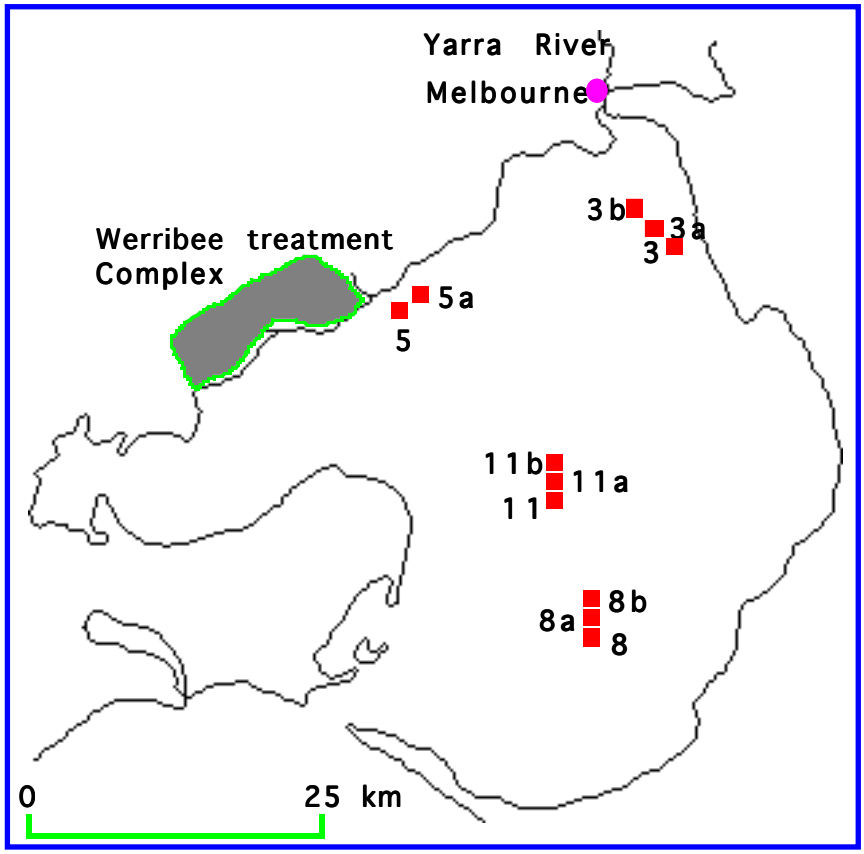


Figure 2: Sampling stations for short time and space scale experiments

2.2. Chlorophyll *a* determinations

2.2.1. Sampling and size fractionation.

Part of the water sample collected from mid-depth at each station was filtered under low pressure through a 0.2 μm pore sized polycarbonate filter (Poretics) which was stored in liquid nitrogen. These filters were used to determine the chlorophyll *a* concentration for the total population using spectrophotometric techniques (see below). For all other depths 2 x125 mL water samples were used to determine the chlorophyll *a* concentration for the < 20 mm fraction and the 0.2-2 μm fraction. This involved pre-filtering one sample through a 20 μm screen whilst the other sample was passed through a 2um polycarbonate filter.. After pre-screening, the remaining cells were collected on polycarbonate filters of 0.2 μm pore size and stored in liquid nitrogen. From March, 1994, onwards a third 125 mL water sample was collected from both surface and bottom and passed through 0.2 μm pore size filters which were stored in liquid nitrogen for determination of the chlorophyll *a* concentration for the total population. Chlorophyll *a* concentrations for the fractions and total population from surface and bottom water were determined using fluorometric techniques.

Chlorophyll *a* concentrations were determined using both spectophotometric and fluorometric techniques. Filters were removed from liquid nitrogen, placed in 100% acetone and allowed to extract overnight at 4 ^{o}C.

Acetone concentrations were adjusted to 90% prior to reading on either the spectrophotometer or the fluorometer. The chlorophyll *a* concentrations for the unfractionated samples collected from mid water were determined using measurements of absorbance at 630, 647, 664 and 750 nm. and the equations of Jeffrey and Humphrey (1975). The fluorescence of these samples was also determined using a Hitachi F2000 fluorometer with excitation wavelength of 436 nm and an emission wavelength of 686 nm. Linear correlations of fluorescence vs chlorophyll *a* concentration obtained from these samples provided the calibration for determination of chlorophyll *a* concentration by fluorescence of extracted chlorophyll *a* for all fractionated and for total population samples from surface and bottom water. The chlorophyll a concentrations for the micro and nano fractions were determined by difference. The total chlorophyll *a* concentrations for surface, mid and bottom water for each field trip were pooled and used to calibrate the SeaTech fluorometer for *in situ* estimates of chlorophyll *a* from fluorescence.

The techniques for determining chlorophylls and the relationship between chlorophyll *a* and fluorescence raised some interesting procedural problems. These are discussed in some detail in Section 3.

2.3. Carbon fixation

All carbon fixation experiments were carried out using water collected from mid depth at each station. Sub-samples of 150 mL were poured into 150 mL polycarbonate bottles that had been washed using the method of Fitzwater *et al.* (1982). To each bottle was added 1 mL of $H^{14}CO_3$ solution that contained approximately 40 x 10^6 d.p.m. per ml^{-1}. When P vs I curves were to be constructed a total of 54 bottles were incubated for a station. Of these, 9 bottles (6 light and 3 dark) were incubated at each of 6 light intensities. At stations where only light saturated carbon fixation rates were to be determined, just nine bottles (3 dark, 6 light) were used. Incubations were for four hours in an incubator cooled with sea water. Light was provided by True Light fluorescent tubes with intensity modified by nickel screens (Buckbee-Meers). After incubation, samples were filtered at low pressure. Two light bottles and one dark bottle were used for the total population, the < 20 μm fraction and the 0.2-2 μm fraction with fraction separation carried out as described for chlorophyll *a* (Section 2.2.2). After filtration, filters were placed in scintillation vials, 200 ml of 1 N HCl added to each vial and the vials allowed to stand overnight (Lean and Burnison 1979) before scintillation fluor was added (Ready Safe, Beckman). The activity in the particulate fraction was determined using a Beckman scintillation counter. The amount of carbon fixed at each light intensity was determined using the equations of Parsons *et al.* (1984). Chlorophyll *a* specific carbon fixation rates were used to fit the exponential function (Jassby and Platt 1976) with no correction for photoinhibition.

Daily estimates of *in situ* carbon fixation were made using an exponential function relating chlorophyll *a* concentration, incoming irradiance and light attenuation through the water column. Incoming global irradiance from a weather station at Laverton, on the shores of Port Phillip Bay, was provided by the Bureau of Meteorology. These data were 30 minute averages of incoming irradiance in W m^{-2} .The data were converted from total (global) energy to photosynthetically active radiation (400-700nm; PAR) by multiplying by 0.42 (Jitts *et al.* 1976) and this value was then converted to total quanta by multiplying by 2.5 x 10^{18} (Collos *et al.* 1992).

Depth profiles for light were measured with a Licor quantum sensor system which had an underwater sensor, deck sensor and data logger. This equipment provided simultaneous measurements of both surface PAR and the amount of PAR reaching a particular depth. The ratios were used to obtain regressions of the proportion of incoming irradiance as a function of depth (Kirk 1983). The amount of light reaching 0.5 m depth intervals was estimated from these regressions and incoming irradiance. Areal estimates of *in situ* production were obtained by summing estimates for 0.5 m depth intervals. Chlorophyll *a* for these calculations was estimated as the average of either surface + mid or mid + bottom values for each depth interval. For monthly estimates of primary productivity, light values for 2 weeks prior and 2 weeks after the cruise were used.

On each cruise, data to construct P vs I curves were collected from 4-5 stations. For stations where only light saturated carbon fixation rates were determined, estimates of alpha were derived from the average of values obtained for each cruise. In a number of cases, samples were lost or data deemed unreliable. Data were deemed to be unreliable when either the difference between duplicate bottles was greater than the lower value obtained or when visual inspection of P vs I curves showed anomalies. In cases where estimates appeared unreliable and chlorophyll *a* concentrations were less than 0.1 mg l^{-1} , minimum values were used. In cases where it appeared that unreliable data were due to some other factor then average values from all stations were used.

3. RESULTS & DISCUSSION

3.1. Notes on the methodology of chlorophyll *a* estimations

This project used both fluorometry and spectrophotometry to determine chlorophyll *a* in the water column. It was important from the outset to establish that these two methods gave comparable data and to estimate the magnitude and sources of error associated with both techniques. To this end we examined critically :

a) the relationship between fluorometric and spectrophotometric analyses of chlorophyll *a in vitro*

b)the reliability of collecting samples for chlorophyll analysis on polycarbonate vs GFC filters

c) the relationship between *in vivo* chlorophyll fluorescence and extracted chlorophyll *a*.

3.1.1. Chlorophyll *a* analysis by fluorometry vs spectrophotometry

Water samples for total and size fractionated chlorophyll *a* for fluorospectrophotometric analysis (125 mL) were calibrated against spectrophotometric samples (500 or 1000 mL) taken on the same field trip. The relationship between measurements using both techniques for these samples was always linear and had an r^2 value greater than 0.99. The limit of detection for this method is approximately 0.01 mg/m^3. Pheophytin was measured spectrophotometrically but this method is prone to high errors particularly when the absorbance for chlorophyll at 664 nm is low. A second method for estimating pheopigment is to determine the acidification ratio of the fluorospectrophotometric samples. All results showed consistently low levels of pheopigments in the samples at all depths.

3.1.2. Membrane filters versus glass fibre filters

This is the first long term study to use polycarbonate filters (pore size 0.2μm) in the collection of chlorophyll samples in Port Phillip Bay. Most previous studies have used glass fibre filters (GF/C), which have a nominal pore size of 1.2 μm. At the same time as we collected samples from three depths, an integrated sample from all depths was collected and filtered on a GF/C filter for the Nutrient Task N2.2. When results from both tasks were compared in early 1994, it was noted that the Nutrient Task values for chlorophyll *a* were consistently lower than our values. In April 1994, a small scale comparison from a pooled sample was performed using polycarbonate and GF/C filters with three filters of each type. This experiment showed an average underestimate of total chlorophyll of 30% when using GF/Cs. A later comparison of the data from the six coastal stations (1-6) from the whole of 1994 (n=76) between the depth averaged chlorophyll concentration of our three samples against the integrated value from the N2.2 Task again shows a consistent underestimation of total chlorophyll by the GF/C method of 32%. Observation of the correlation between the two sets of data shows a high r^2 value of 0.929 and a slope of 1.06. In addition, the regression line does not go through zero but shows the polycarbonate filters to produce a chlorophyll concentration of approximately

0.5 mg/m^3 that does not appear using GF/Cs. Some of the possible causes for the discrepancy between the two techniques could be:

a) Some loss of the pico-fraction through the GF/C filters due to their larger and more variable pore size.

This is the most probable cause of the major part of the discrepancy. We know that, on average, 32% of the phytoplankton are in the size range 0.2-2μm and a high proportion of these cells is likely to be able to pass through a GF/C filter. However, even if all of these cells passed through this would not account for all of the 'missing chlorophyll' because their chlorophyll *a* equivalent is only 0.47 mg/$m^{3.}$

b) A methodological error in the sample collection and processing.

Although possible, this is unlikely. Both the laboratories have routinely determined chlorophyll concentrations from microalgal samples for more than ten years. Any consistent error in either a volume size or equation variable is most likely to cause a change in the slope of the correlation between results from both laboratories. In addition when the two laboratories each independently measured the same standard extract (US Environmental Agency, Cincinnati) from a mixed chlorophyll sample, both obtained the same results.

c) Lower extraction efficiency in the GF/C filters.

There are a number of microalgal species that are known to be resistant to organic solvent extraction of chlorophyll. The efficiency of extraction of these species is often dependent on their pre-treatment and length of exposure to the solvent. Both laboratories used 90% acetone as the extracting solvent. Because GF/C filters are quite bulky, it is normal procedure to either sonicate or macerate the filters in order to disrupt or free embedded algal cells from the filter matrix. This procedure can in some cases be counterproductive because localised heating as well as exposure to light can degrade extracted chlorophyll. Our procedure included only a short, low power, sonication step because the polycarbonate filters have a flat surface and are more readily permeable to acetone.

A long term comparison of these two filter types being run by the N2.2 task team may clarify some of the probable causes of the differences between these two data sets.

3.1.3. Analysis of the errors associated with chlorophyll estimates

Effects due to Size Fractionation Procedures

Size fractionated concentrations of chlorophyll *a* were calculated by subtraction. Three chlorophyll values were obtained for each analysis by filtering three separate sub-samples onto a 0.2 μm polycarbonate membrane filter. Two of these samples were pre-filtered through a either a 2 or 20 μm filter to obtain a "> 2 μm" and a "> 20 μm" value. This method assumes that there is a low level of error associated with the sub-samples either from heterogeneity of the chlorophyll within the bulk sample or arising from the filtering and extraction process (see reproducibility below). In addition, there should be little interference from retention on the filter apparatus or cell breakage. A test of this method

was performed by extracting the chlorophyll retained on the pre-filters. Nine separate trials were run on field samples in July 1994. More than 90% of chlorophyll retained was present on the pre-filter.

Reproducibility

During a field trip in January 1995, three 125 mL. sub-samples were obtained from each bulk sample at the surface, middle and bottom at two stations in the vicinity of Hobsons Bay. All sub-samples were filtered for total chlorophyll extraction and analysed fluorometrically. The mean, standard deviation and coefficient of variation for each of the six bulk samples were calculated. Standard deviation, as a percentage of the mean for all the samples, was less than 2%, indicating that in 95% of cases the error of estimation is less than ± 4%.

3.1.4. Total Chlorophyll *a* measured by *in-situ* fluorescence (F).

In-situ chlorophyll *a* fluorescence (F) was measured by depth profiling in conjunction with parallel acetone extracted chlorophyll *a* samples from surface, middle and bottom waters at all stations every four weeks from 1/3/94 until 6/12/94. A faulty cable prevented fluorescence readings during the two field trips in June 1994 but, in total, 241 separate calibration values were obtained. Correlation between F and extracted chlorophyll *a* for all values and each depth are presented in Figure 3a. When all depths are considered together, chlorophyll *a* accounts for 71% of the change in F. The results however, indicate that when F is greater than 900 there is a large amount of variation in F, not associated with the chlorophyll concentration in extracts. Selection of values less than 900 and the exclusion of a single outlier retains more than 96% of samples with an r^2 value of 0.87.

Effects of sample depth

Sample depth had a direct effect on both the slope of the correlation between chlorophyll *a* in extracts and F, and the error associated with the estimate of chlorophyll from F. The slope of the correlation represents a mean value of fluorescence output per chlorophyll *a* (F/Chl) for that data set. As depth increases F/Chl increases from 168 at the surface to 207 in bottom waters, an increase of 23% (Figure 3b-d). During the course of this study there appeared to be a constant background fluorescence, not associated with chlorophyll *a*, of approximately 36 units. This background fluorescence is most probably due to an ubiquitous component of the water column which fluoresces in the range for chlorophyll fluorescence.

Errors of the *in-situ* estimates of chlorophyll *a* using F can be calculated using the residuals of the correlations. Histograms of the percentage error of the estimate from the measured value are presented in Figure 4. Errors associated with the estimate of chlorophyll from F are consistently larger in surface compared to middle or bottom waters. In surface waters 57% of cases have an error of greater than ±20% of the estimate compared with less than 20% of cases at the other two depths. Errors of more than ±50% of the estimated value occur in less than 5% of samples for all depths.

The F/Chl ratio as mentioned above is commonly used for calibration of field estimates of chlorophyll *a* from *in-situ* readings of F. This ratio is known to be rapidly affected by environmental factors such as light or fluctuations in nutrient supply (Roberts and Beardall, unpublished). However, if corrections can be made for these effects, F/Chl can be used to infer the nutrient status of the measured phytoplankton population. (Kiefer, 1973); (Cleveland & Perry, 1987); (Wood & Oliver, 1995), (Kolber et al., 1990); (Falkowski & Kolber, 1995); (Greene & Kolber, 1994).

As species composition can also have a significant effect on this ratio, inherent in this interpretation is the assumption that the relative population species structure is not significantly different in the samples. F/Chl ratios for individual samples ranged from 80 to over 700 with a mean of 225 and the majority of values normally distributed between 100 and 350. Values outside this range were designated outliers in a box plot of all values (Figure 5b). The ranges were narrower for separate depths (Figure 5c and d).

Effects of Light

Light at ambient levels can severely depress F/Chl in phytoplankton *in-situ* (Falkowski & Kolber, 1995) . Reduction in F/Chl induced by light is referred to as "quenching" and generally has two components both of which are readily reversible if light is reduced. Photochemical quenching is rapid and subsides in seconds. Non-photochemical quenching on the other hand dissipates over tens of minutes. Ambient light (measured as PAR) was recorded at all stations within 20 minutes of the time of sampling. Light intensity over the year ranged from zero to greater than 1800 μEin m^2 s^{-1} at the surface (0.5 metres) and from zero to 650 μEin m^2 s^{-1} in bottom waters. A decrease in F/Chl was significantly correlated to increasing ambient light ($P< 0.01$; Figure 5b). Mean F/Chl without the effect of light was 244 decreasing by 0.057 units per μEin m^2 s^{-1}. Ambient light however only accounted for 25% of the measured variation in F/Chl. Although we were only able to measure ambient light levels at the time of sampling it is known that previous light history can reduce F/Chl for up to an hour after exposure. Ambient light below 200 μEin m^2 s^{-1}appeared to have little effect on F/Chl, the mean of values below this limit being 241, in close agreement with the light independent value. This group of values (n=143) was used to investigate other possible factors affecting this ratio.

Effects of Cell Size

Use of the F/Chl ratio for biomass estimations is often confounded by the fact that different species have been shown to exhibit a large range of F/Chl values. These changes have sometimes been related to different accessory pigments and/or morphological variations in chloroplast structure. In addition it is postulated that smaller sized cells will have higher F/Chl ratios as there is a reduced likelihood of emitted light being reabsorbed by internal cellular components. This has been referred to as the 'packaging effect'. Alpine and Cloern (1985) measured F/Chl ratios of three size fractions over an annual period in two embayments of San Francisco Bay. These workers separated the phytoplankton into 'net' >22μm, 'nano' 5 to 22μm and 'ultra' 0.45 to 5μm by filtration, and measured dark-adapted fluorescence and extracted chlorophyll. Regression of fluorescence against chlorophyll showed decreasing F/Chl with increasing size. Overall, relative F/Chl was in the ratio 1:2:4 for 'net', 'nano' and 'ultra' plankton respectively. This size dependence of F/Chl has also been demonstrated in a highly oligotrophic oceanic environment (602).

Our results did not directly measure fluorescence output for each size fraction but the percentage contribution of each fraction to particular samples ranged from 10 to 70% in the pico and zero to 80% in the micro (see Section 3.2.3). A plot of mean values of the size fractions as a portion of total chlorophyll versus F/Chl output shows a clear trend of increasing F/Chl with decreasing average cell size (Figure 6). As the mean percent contribution of the pico fraction increases from 25% to 45% of total chlorophyll relative fluorescence output more than doubles.

Effects of cell size should be considered when interpreting broad scale chlorophyll concentrations calculated from *in-situ* chlorophyll fluorescence data. Errors could be manifested in two ways. The higher fluorescence output from smaller cells may either mask or magnify changes in total extractable chlorophyll if the relative concentrations of the size classes also change along a transect. Our extracted station data have shown that the relative concentration of pico plankton can vary from less than 10% to greater than 60% of total chlorophyll on a single field trip (Section 3.2.3, Figure 9). The effect of light on F/Chl has been previously discussed for total chlorophyll. However, the magnitude of short term quenching of chlorophyll fluorescence may be different for each size class. Photochemical quenching which is highly related to the photosynthetic capacity (Pmax) of the algae will differ among classes with season and station. Non-photochemical quenching has been shown to induce rapid morphological changes in diatom species where chloroplasts shrink and move to the distal ends of the cell (Kiefer, 1973), this phenomenon can lead to a 60% reduction in F/Chl in less than 30 mins. The response of the smaller sized cells to non-photochemical quenching is likely to be similar, although the magnitude of the change may differ.

Effects associated with station

The nine stations sampled in this study range in depth from 8 to 22 metres, with the three central Bay stations being at least twice the depth of the three most northerly coastal stations (Sandringham, Williamstown and Werribee). The latter group of stations could also be considered to represent examples of stations in closest proximity to the major point sources of nutrient inputs to the Bay, viz., the Yarra River and the Werribee Treatment Plant (WTP). The potential effect of these sources on F/Chl could be two fold. Firstly, the phytoplankton close to these sources would be expected to be less nutrient limited for some or all of the year, this would tend to reduce F/Chl. Secondly, the relative proportion of species present may change in response to nutrient enrichment. If this favoured bigger organisms (i.e. diatom and dinoflagellate species) the F/Chl would also tend to be lower.

In order to compare the potential effect of nutrients on F/Chl in these groups of stations it was necessary to control for the effect of recent ambient light history. Samples from 8-10 metres and with an ambient light of less than 200 μEin m^2 s^{-1} were selected (Figs 5 c,d). It is assumed that the relative mixing rates from these depths to the surface are similar in both groups of stations. Mean F/Chl for these groups were 256 and 222 for central and coastal groups respectively, with standard deviations similar at 30 and 27 units of F/Chl. A Mann-Whitney U-test for these two groups showed them to be significantly different at the 0.001 level. Comparison of the mean value of F/Chl for Autumn, Winter and Spring 1994 show a consistent difference between these two groups across all seasons. Trends in mean F/Chl show winter minima for both groups.

Increases in F/Chl with nutrient limitation have been demonstrated in a number of field and laboratory studies (Kiefer, 1973; Wood & Oliver, 1995; Kolber et al., 1990; Falkowski & Kolber, 1995) . The difference in F/Chl between the two groups of stations

analysed indicates that the phytoplankton in coastal group stations are on the whole less nutrient limited. As nutrient status is often a function of flux of a nutrient rather than ambient concentration it would also be expected that measurements of carbon fixation would be higher on a per unit chlorophyll basis at the coastal stations. The use of F/Chl as an indicator of nutrient stress may be confounded in this study by the effect of ambient light on many of the samples collected. However, the indications are that this parameter could be used, with appropriate methods, to give a quick and easily collected information on phytoplankton physiological status over broad areas.

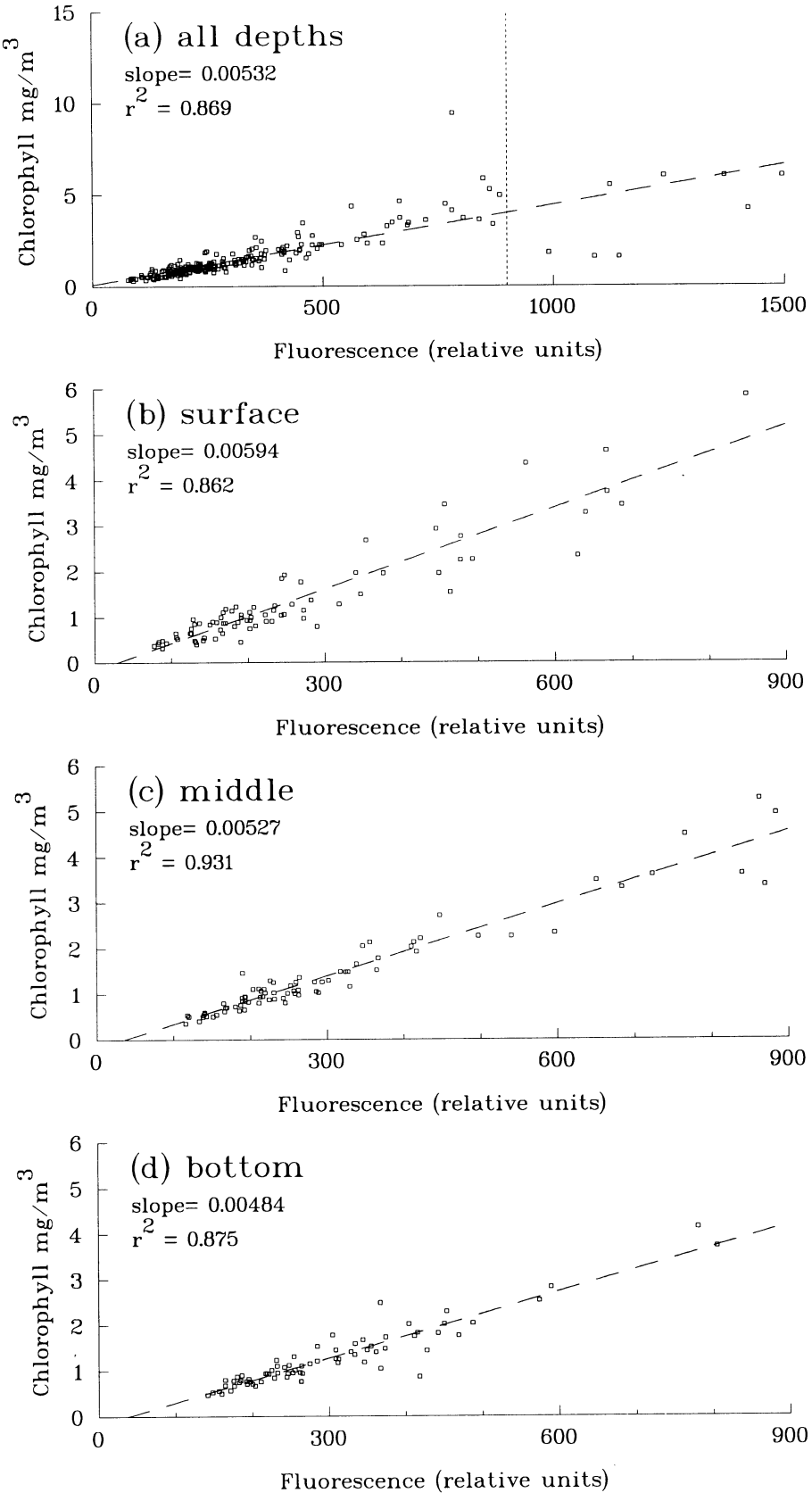
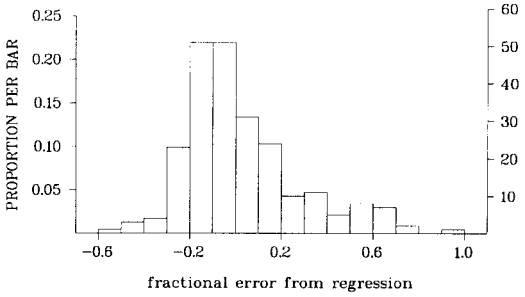


Figure 3: The relationship between extracted chlorophyll *a* and *in vivo* chlorophyll luorescence. [A] Data for all stations and depths, [B]-[D] samples with F<900. [B] surface waters, [C] mid-depth and [D] bottom waters.

(a) All Depths

±20% error= 67% of cases

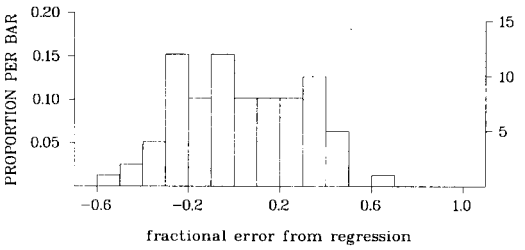
±30% error= 80% of cases



(b) Surface

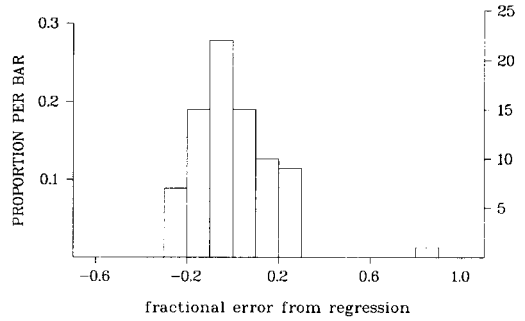
±20% error= 43% of cases

±40% error= 80% of cases



(c) Middle

±20% error= 80% of cases



(d) Bottom

±20% error= 80% of cases

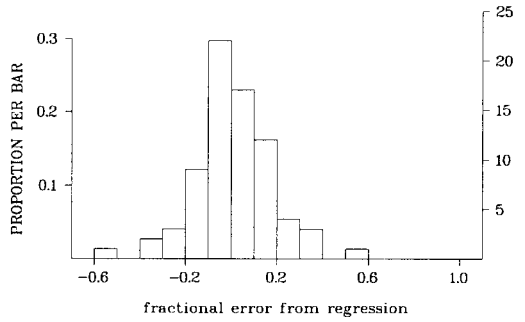
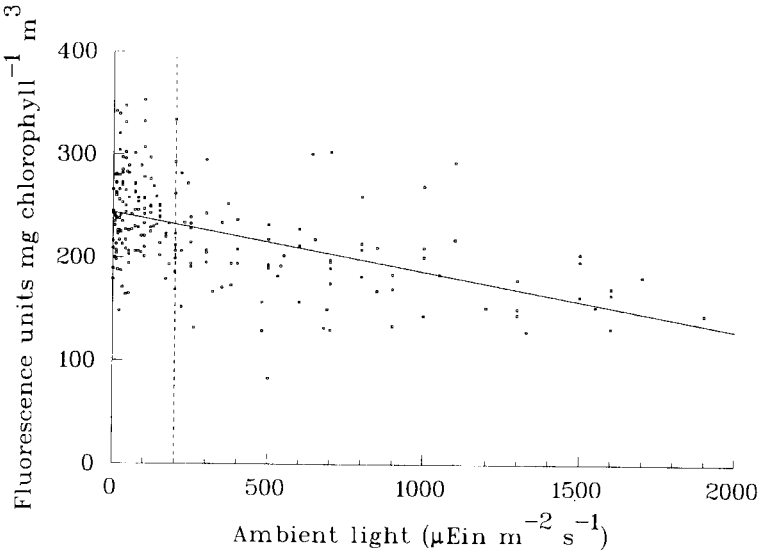
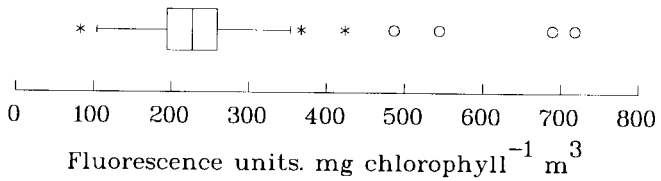


Figure 4: Histograms of the error associated with determining chlorophyll *a* concentrations from i*n vivo* flourescence. Errors are consistently greater in surface waters than in mid-depth or bottom waters.

(a) All stations

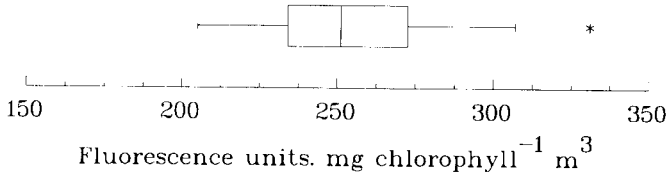


(b) All stations



(c) Deep

stations 8,9&11
middle waters
light<200µEin



(d) Shallow

stations 3,4&5
bottom waters
light<200µEin

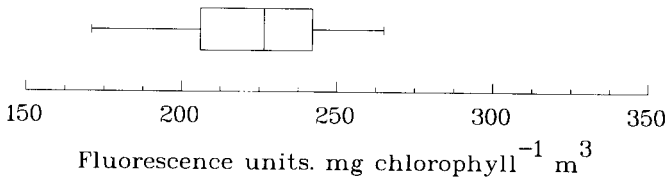


Figure 5: Graph [A] shows the regression between *in vivo* fluorescence per unit chlorophyll and photon flux at the depth of sampling. [B], [C], [D] are box plots of *in vivo* fluorescence per unit chlorophyll for all stations, deep stations and shallow stations respectively. Data are from March to December 1994 and in [C] and [D], data are taken from sites with photon flux <200mEin.m^{-2}.s^{-1}

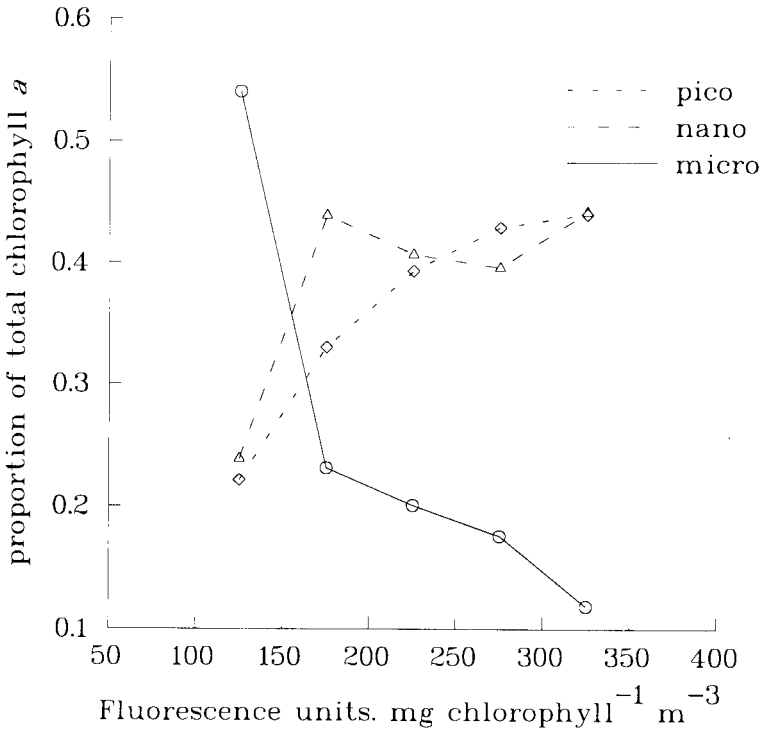


Figure 6: The proportion of chlorophyll *a* associated with each of the phytoplankton size fractions as a function of the *in vivo* fluorescence per unit chlorophyll (F/Chl). Low values of F/Chl are associated with a high proportion of microphytoplankton biomass and low proportions of nano and pico plankton.

3.2. Seasonal changes in phytoplankton biomass (chlorophyll *a*)

Extracted total chlorophyll *a* values for each station, for the period February, 1994, to the end of March, 1995, and all depths are plotted in Figure 7. It was possible to designate certain stations as characteristically showing high chlorophyll concentrations in the water (see 3.2.3, below). These were also those stations designated above as "coastal" stations. Importantly many of the stations in the central, low chlorophyll, group show a high correspondence in their pattern of change in chlorophyll *a* with time. Some of the coastal group of stations show some similarity to this pattern, however others appear to be unique. There are often major differences in chlorophyll *a* with depth at any one sampling time (see Section 3.4 below). These differences with depth are frequently as large as the change in average water column concentration between trips, and/or the difference between station chlorophyll *a* concentrations on a particular date.

The three central stations and Blairgowrie formed a group with very similar absolute values and seasonal trends in chlorophyll *a*, particularly from autumn 1994 to summer 1995. All these stations show an approximate doubling of chlorophyll during autumn 1994, peaking at around 2 mg/m^3 at the start of winter. Concentration of chlorophyll then declined over the following four months, reaching a minimum of less than 1 $mg.m^{-3}$, at all stations in either September or October. This period of decline is also characterised by very little apparent difference in total chlorophyll *a* with depth. During the next three months (late spring to early summer) chlorophyll concentration increases, accompanied by a return of differences with depth which commonly can be as large as month to month changes in average water column values.

Both Sandringham, Clifton Springs and Wooley Reef stations show some similarity to this pattern with chlorophyll rising from a low base during autumn. Wooley Reef loses half this chlorophyll during winter and spring but whilst there is a large level of variation in month to month concentrations, there is no apparent increase in concentration during the following summer. Autumn increases in chlorophyll at Sandringham and Clifton Springs continue into winter culminating in a rapid decline in concentration at all depths in late winter or early spring. Concentrations again increase during the summer of 1995. Differences with depth appear much more prevalent over all seasons at these three station compared to the central and southern stations.

Williamstown and Werribee have unique patterns, although both seem loosely correlated in autumn and winter with a general trend of decreasing concentration during spring Williamstown values then begin to increase leading to a late summer early autumn 1995 maximum. Werribee however experiences a single early spring 'spike' in concentration that does not seem to effect the overall trend of a relatively low constant concentration for the rest of the seasonal cycle.

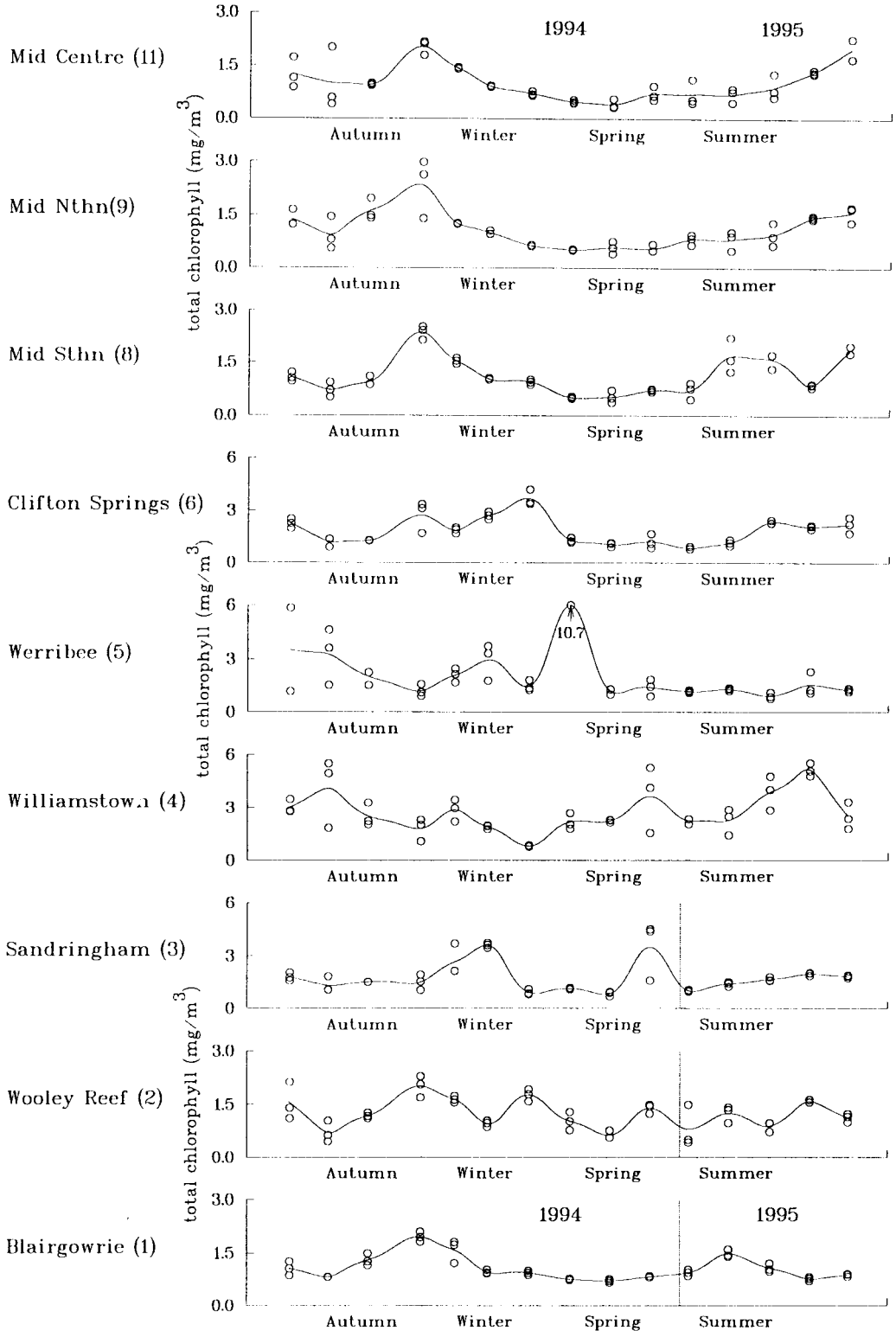


Figure 7: **Seasonal variation in total chloraphyll *a* at different stations in Port Phillip Bay. Each point represents surface, mid or bottom water samples. The line is the distance weighted least square fit to all data points.**

3.2.1. Contribution of size fractions to total chlorophyll.

Table 2 summarises the three size fractioned chlorophyll *a* statistics for all the stations together over an annual period. Mean chlorophyll *a* for the three groups only differs by 24% between the groups but the median value for the micro fraction is less than half of the two smaller fractions. The overall distributions of each fraction are illustrated as quantile plots in Figure 8 a. The two smaller size fractions have a relatively even distribution of chlorophyll in the lower eighty percent of values (0.15 to 0.75 mg/m^3) the highest 20% of values then increase exponentially in concentration. The micro fraction as discussed in the previous section has a significant proportion of undetectable values, but chlorophyll concentrations rise in an exponential pattern from this base. The use of the mean values as a descriptor of central measure in this distribution may be misleading as a few high values have a large amount of influence on this parameter. The median value generally gives a more accurate indication of these distributions. All three fractions can be transformed to a near normal distribution by converting to the log base 10 (Figure 8 b & c).

Table 2: Annual Size fractioned Chlorophyll *a* Statistics, March 1994 to February 1995.

Size Fraction (microns)	Mean (mg/m^3)	proportion of total	median (mg/m^3)	range (mg/m^3)
Micro (>20 μM)	0.47	29%	0.20	0.0 - 8.14
Nano (2 - 20 μM)	0.62	39%	0.45	0.0 -4.36
Pico (0.2 - 2 μM)	0.51	32%	0.43	0.1 - 2.57

Figure 9a presents the average contribution of each size fraction to the total biomass for each station over the annual period March 1994 to February 1995. No single size fraction represents more than 45% or less than 26% of the total chlorophyll at any one station. Differences in station mean annual total chlorophyll values are not correlated with any change in the relative contribution of any particular size fraction. The Williamstown station (4) has on average a higher nano fraction biomass (46%), however all the other stations show relatively similar proportions of each size fraction contributing to the total chlorophyll. Overall, the relative ranking of the stations in terms of mean and median total chlorophyll *a* concentration (Table 3) is little influenced by the relative proportion contributed by the different size fractions.

Stations can be split into two groups on the basis of the annual average total and size fractioned chlorophyll *a* concentrations, the minimum and maximum recorded chlorophyll *a* and the statistical probability that the population of values collected for each size fraction are different (see below). Figure 9b groups the stations into two concentration classes by setting a limit of at least one size fraction having an average annual biomass of at least 0.5 mg/m^3. This analysis conveniently groups the stations into two broad regional areas. Stations that have on average higher concentrations of chlorophyll *a* are all found in the coastal northern and western portion of the Bay (stations 3,4,5 & 6), the remaining stations (1,2,8,9 & 11) are either located in the central Bay or the southern and south-eastern coastal region. It should be noted that this grouping also sub-divides the stations on the basis of depth with the central stations being between 12 and 22 metres deep and the coastal stations being 8 to 11 metres in depth (Table 3, see Section 3.6).

Although the average annual contribution of each size range to total chlorophyll *a* can often be similar at individual stations, the relative seasonal contribution, over an annual cycle, is significantly different at a number of stations (Figure 10). This is particularly apparent when the two sectors (coastal and central) are compared (Figure 9b). Bay wide,

the micro population shows a highly seasonal pattern of chlorophyll concentration with maximum values occurring in autumn or winter and minimum values in spring. The chlorophyll values collected in September 1994 at the Werribee station, when total chlorophyll concentration exceeded 8.5 $mg.m^{-3}$ at all depths, are excluded from this analysis. The F/Chl values collected at the time indicate that at least some of this material may be due to a recent benthic resuspension event.

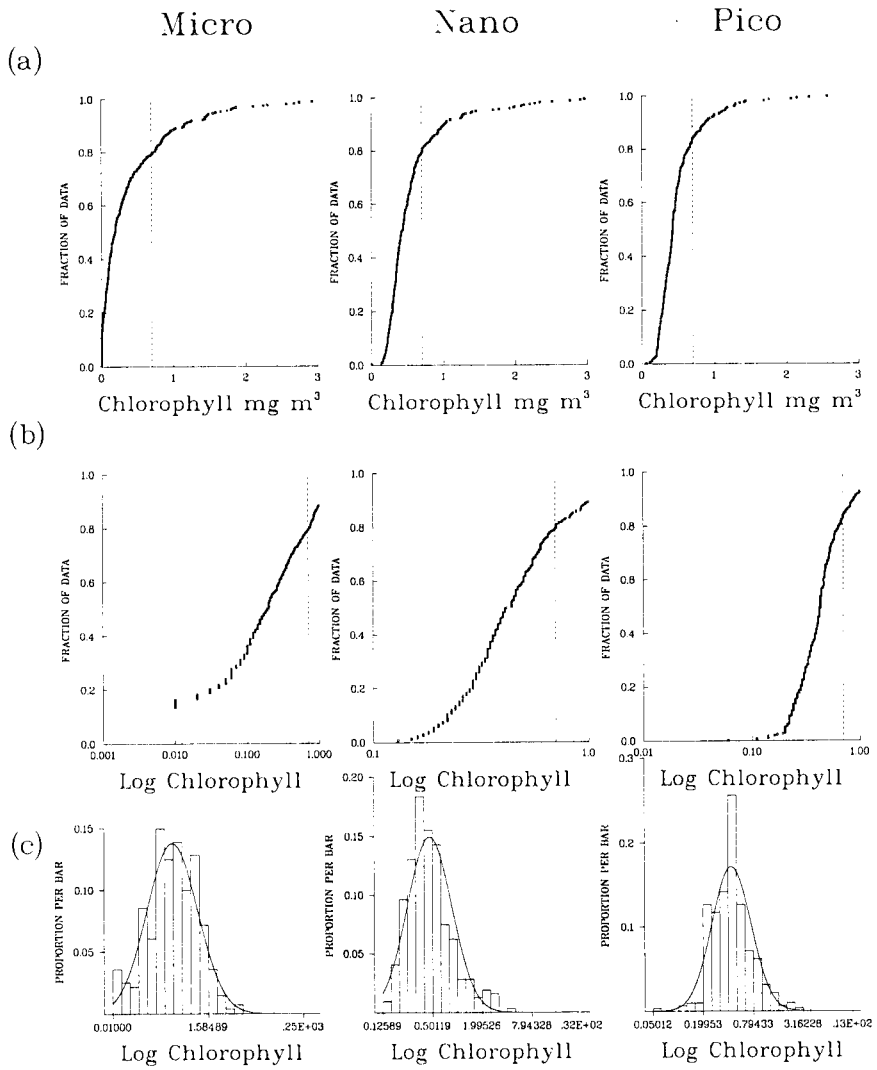


Figure 8: [A] Quantile plots of chlorophyll *a* associated with each size fraction. Note that in a significant number of occasions, chlorophyll levels are extremely low to undetectable in the micro fraction compared to the other two size fractions. [B] and [C] Log_{10} transformed quantile plots and frequency distributions of the chlorophyll data used in [A].

The range of seasonal average standing stock in the micro fraction is greater than fourfold in both sectors. The micro population is the primary size fraction driving seasonal changes in total chlorophyll *a* standing stock, however the nano fraction also follows the general pattern of high autumn values and low spring values in the central group. The coastal stations, in contrast, have a maximum in the nano concentration in summer and a minimum in winter, this pattern is primarily driven by the Williamstown station which also exhibits this pattern for the pico fraction. Seasonal trends in the pico chlorophyll *a* concentration are not common, with this fraction generally constrained within a small range of concentrations most of the time (Figure 8a). On average this size fraction contributes a stable seasonal concentration of biomass as chlorophyll *a* between 0.35 and 0.6 mg.m^{-3} in the central and coastal sectors respectively (Figure 9b).

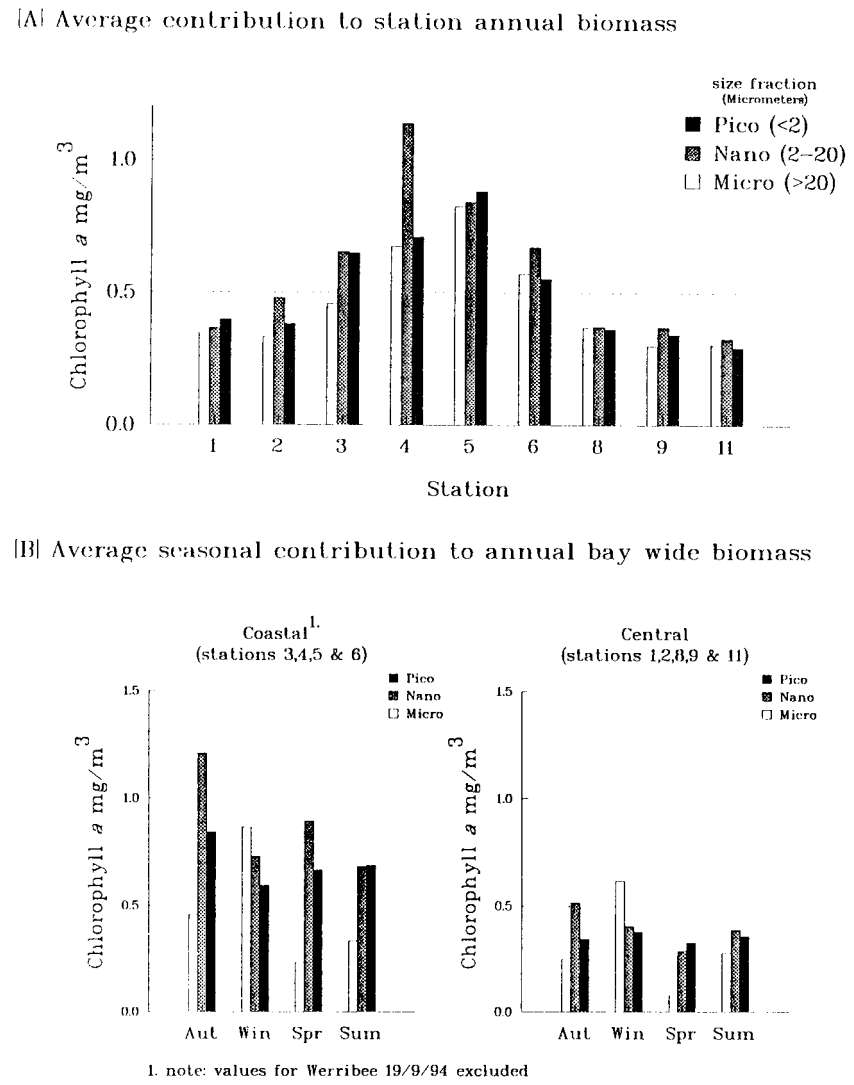


Figure 9: [A]The average seasonal contribution of different size fractions to the annual phytoplankton biomass at each station. [B] The average seasonal contribution of different size fractions to the annual phytoplankton Bay-wide biomass during each season.

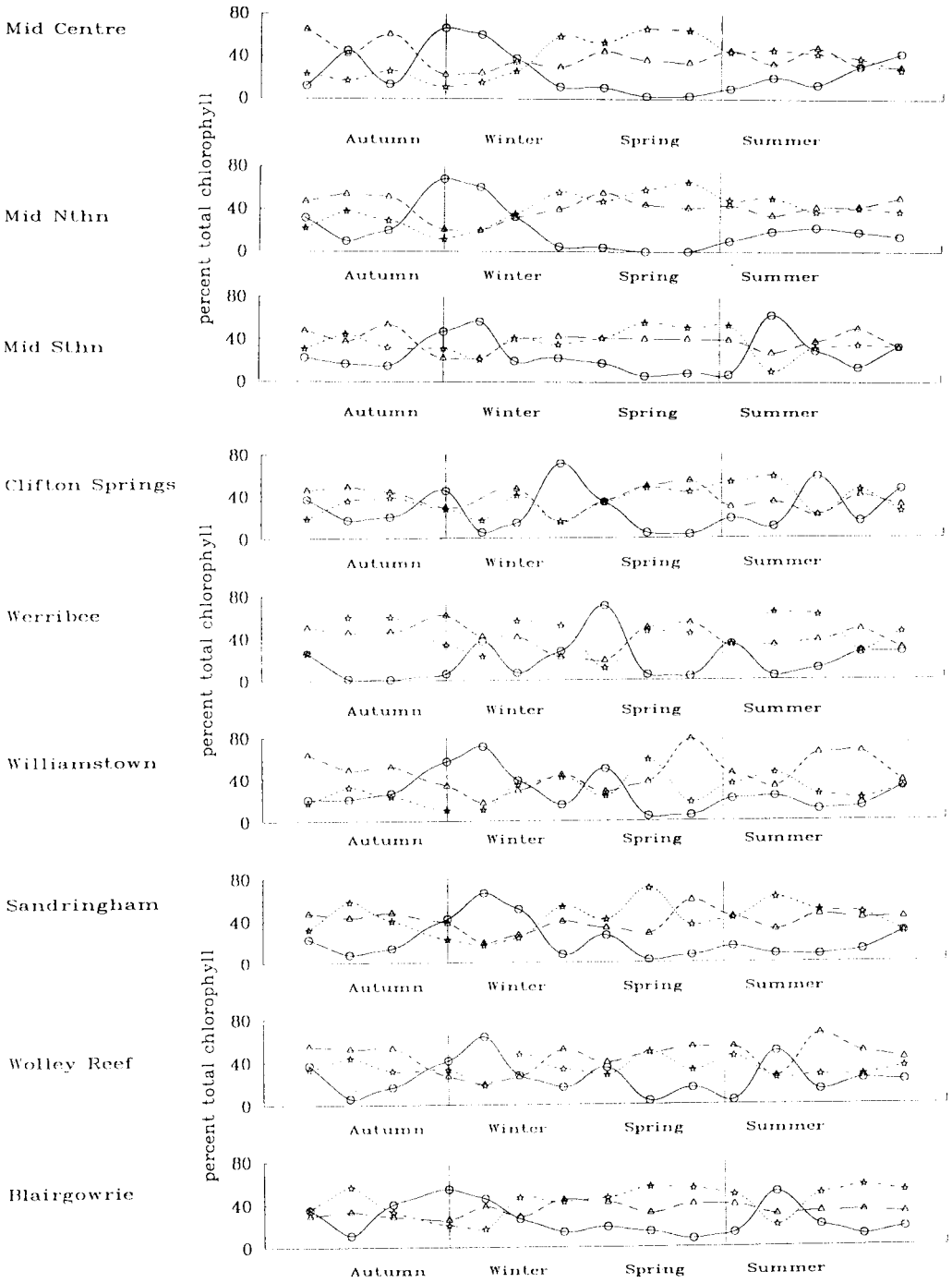


Figure 10: Seasonal changes, by station, in the percentage contribution of each size fraction to the total phytoplankton biomass. Circles=micro; triangles=nano and stars=pico fractions.

Most of the previous results refer to the contribution of the three size fractions to seasonal or station biomass in concentration terms. The relative percentage contribution of each size fraction to water column chlorophyll is also relevant both ecologically in terms of potentially different physiological parameters (respiration, fixation and photosynthetic parameters) and loss factors (such as sinking or grazers) and practically in relation to sampling methods (such as counting and differences in *in-situ* fluorescence output).

Seasonal concentration values of each size class showing a distinct change in the micophytoplankton and relatively small changes in the nano- and picophytoplankton would indicate a change in relative percentage biomass driven by the larger cells.

This pattern has been observed in both coastal and oceanic environments, where a higher percentage contribution of the picoalgae to total biomass has been postulated to be indicative of oligotrophic conditions through enhancement of regenerated production via the microbial loop. Under this hypothesis, increased nutrient supply leads to an increases in larger sized cells which are more likely to either sink or be lost as fecal pellets to the sediments.

This is certainly the case in all the central stations with a strong negative correlation in the relative percent contribution of the picophytoplankton to increasing total chlorophyll (Figure 11). In contrast, the coastal stations only show this correlation at the Sandringham and Clifton Springs stations, both Williamstown and Werribee exhibiting variable contributions of the picoplankton over the whole range of total chlorophyll concentrations. In all but these latter two stations the reduction in percentage contribution of the picoplankton is primarily due to increases in the relative contribution of microalgal biomass. The nano fraction in general maintains a reasonably constant (though variable) contribution, with a median of 40%, over the range of chlorophyll concentrations (Figure 12).

Ecologically speaking the relative percentage contribution of the picoplankton could be used as an indicator of potential loss of nutrients from the water column to the sediments. Wehr and Campbell (1994) demonstrated in a lake mesocosm study that this parameter explained 65% of phosphate and 57% of nitrogen flux to the sediment surface, with a greater relative proportion of picoplankton biomass leading to significantly lower loss rates. If this relationship holds for Port Phillip Bay the overall higher percentage contribution of picoplankton at Werribee and Williamstown will have the effect of uncoupling any relationships between nutrient supply and benthic respiration. Nutrients sequestered in high levels of picoplankton biomass will be more likely to persist in the water column and be transported spatially by physical processes.

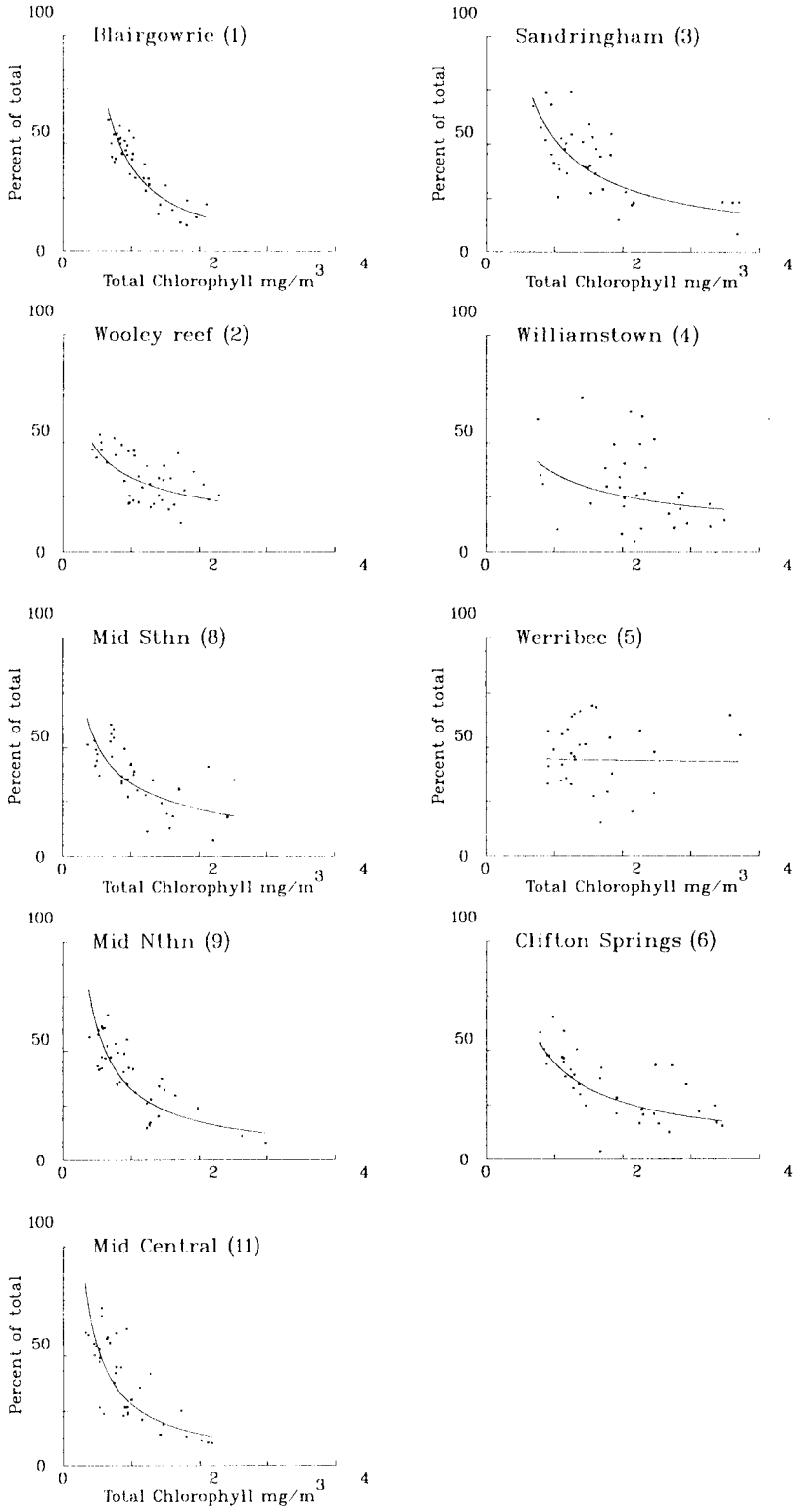


Figure 11: The percentage of total chlorophyll contributed by the picoplankton as a function of total phytoplankton chlorophyll found at each station.

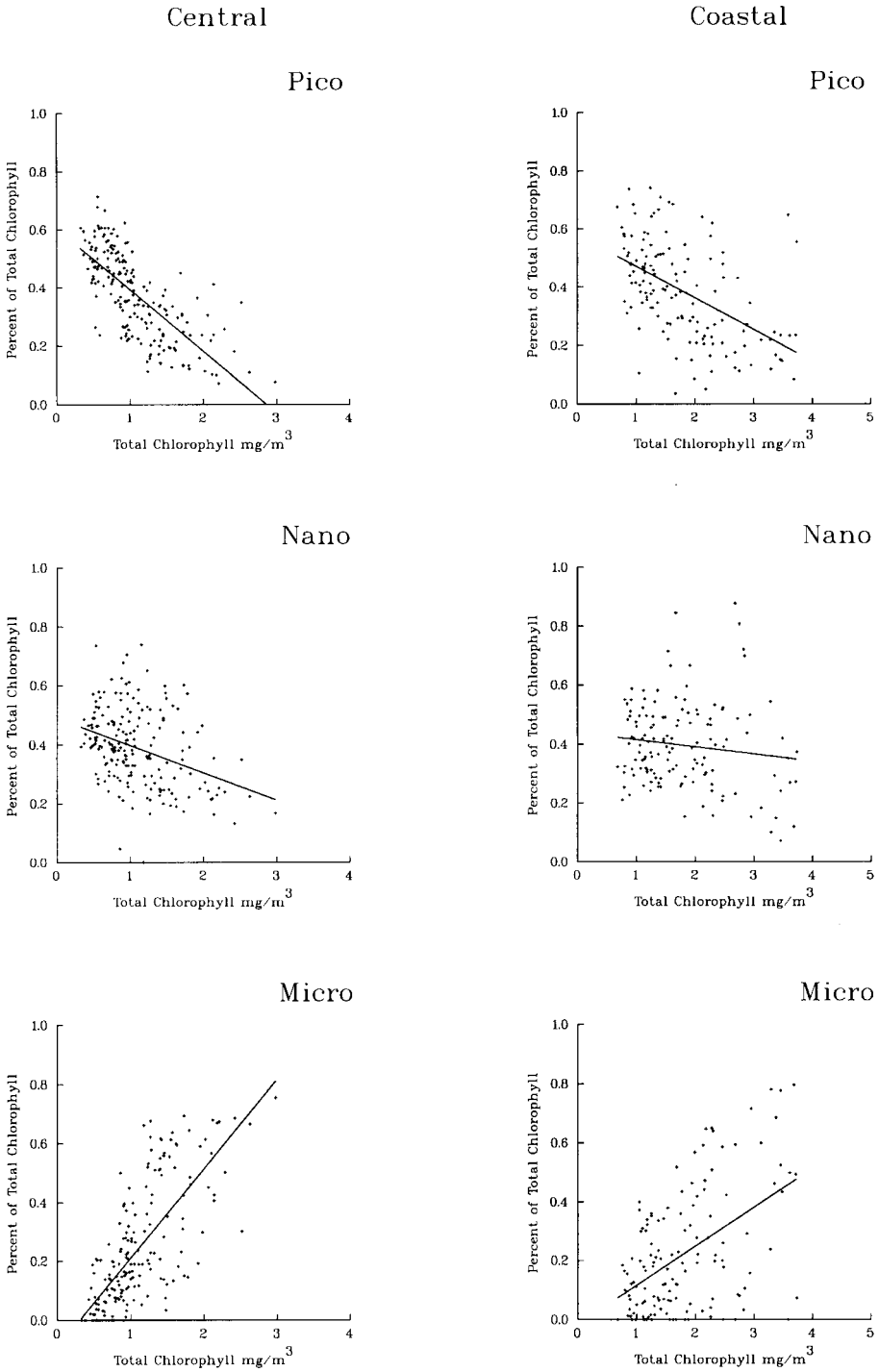


Figure 12: The proportion of chlorophyll *a* found in each size fraction as a function of total chlorophyll *a* concentration. in the water column. Data have been divided into sites characteristically showing "low" or "high" chlorophyll concentrations i.e central or coastal stations. Note that increasing chlorophyll *a* concentrations are associated with an increasing proportion of biomass in the micro fraction and a decreasing proportion of biomass in the pico fraction.

3.3.Spatial variation in phytoplankton biomass

3.3.1. Station statistics

Station annual mean chlorophyll *a*, for the period 1/3/94 to 4/2/95 (13 field trips), varied by almost three fold from 0.93 to 2.70 mg.m^{-3} (Table 3). Individual samples collected range from 0.32 to 11.91 mg.m^{-3} with a mean value of 1.58 mg.m^{-3} and a median of 1.26 mg.m^{-3}. Chlorophyll *a* concentrations in PPB very rarely fall below 0.4 mg.m^{-3} and more than 50% of values fall with the range of 0.5 to 1.5 mg.m^{-3}.

Stations with higher mean chlorophyll *a*, typically show higher monthly values throughout the year, as well as an increase in the occurrence and relative frequency of significant shifts in chlorophyll *a* concentration over weeks to months. This 'spiking' (changes of average water column concentration of greater than 1 mg.m^{-3} over a four week period) is frequently associated with significant differences in the concentration of chlorophyll *a* with depth. There does not seem to be any correlation of these 'spikes' between stations at specific times or with particular seasons, suggesting that they are driven by localised conditions in each area.

Table 3: Annual Total Chlorophyll *a* by Station, March 1994 to February 1995.

Station (No.)	depth (m)	Mean (mg.m^{-3})	standard deviation	median (mg.m^{-3})	range (mg.m^{-3})
Mid Central (11)	21	0.94	0.50	0.85	0.32-2.18
Mid Nthn. (9)	22	1.04	0.57	0.92	0.40-2.98
Mid Sthn. (8)	22	1.10	0.55	0.96	0.37-2.53
Blairgowrie (1)	17	1.10	0.38	0.97	0.65-2.10
Wooley Reef (2)	12	1.21	0.48	1.17	0.42-2.29
Sandringham (3)	10	1.76	1.02	1.52	0.68-4.48
Clifton Spring (6)	10	1.81	0.88	1.45	0.79-4.19
Williamstown (4)	11	2.56	1.17	2.28	0.76-5.50
Werribee (5)	8	2.71	2.88	1.53	0.76-11.91
Average	15	1.58	1.34	1.24	0.32-11.91

3.3.2. Grouping of stations characterised by high or low biomass

Maxima and minima in biomass

Tables 4 and 5 list the 10 highest (greater than 3.5 mg.m^{-3}) and 10 lowest (less than 0.5 mg.m^{-3}) values of total chlorophyll *a* for the annual period march 1994 to February 1995. Only the single highest or lowest depth value from any one sampling trip is presented. These values represent the highest and lowest values recorded from all stations and depths. Absolute and percentage contributions of the three size fractions of chlorophyll *a* are also presented. No station is represented in both the highest 10 and lowest 10 values. Blairgowrie (station 1), is the only station without a representative value in either the top or bottom 10 values.

Table 4: Highest occurrence of Chlorophyll *a* at a Station, March 1994 to February 1995.

Station (No.)	date -depth	total ($mg.m^{-3}$)	micro (% of total)	nano	pico
Werribee (5)	13/9/94-surface	11.91	75	14	11
Williamstown (4)	27/3/94-surface	5.5	25	50	23
Williamstown (4)	8/11/94-middle	5.26	3	83	14
Williamstown (4)	31/1/95-surface	4.77	13	63	24
Werribee (5)	27/3/94-surface	4.63	3	42	56
Sandringham (3)	8/11/94-middle	4.49	8	65	27
Clifton Springs (6)	15/8/94-bottom	4.19	66	20	15
Werribee (5)	19/7/94-surface	3.73	7	37	55
Sandringham (3)	19/7/94-bottom	3.71	49	27	24
Sandringham (3)	26/6/94-bottom	3.68	80	12	8
Average			33%	41%	26%

Table 5: Lowest occurrence of Chlorophyll *a* at a Station, March 1994 to February 1995.

station (No.)	date -depth	total ($mg.m^{-3}$)	micro (% of total)	nano	pico
Central mid (11)	11/10/94-surface	0.33	0	40	60
Central South (8)	11/10/94-surface	0.37	0	48	52
Central North (9)	11/10/94-middle	0.40	0	41	59
Wooley Reef (2)	5/12/94-surface	0.43	7	46	47
Central mid (11)	13/9/94-surface	0.43	11	39	50
Central mid (11)	5/12/94-surface	0.46	2	42	56
Central mid (11)	2/1/95-surface	0.47	0	46	54
Central South (8)	5/12/94-surface	0.47	0	48	52
Central South (8)	13/9/94-surface	0.48	19	39	42
Central North (9)	8/11/94-surface	0.48	0	40	60
Average			4%	43%	53%

High values occur at stations close to the shore in the North and West (Werribee, Williamstown, Sandringham and Clifton springs) whereas low values occur in the central and south-eastern portion of PPB. Highest values occurred in surface waters in five out of ten occasions, do not appear to be correlated with certain times of the year, and show differing patterns in the relative contribution of the size classes to the total. Lowest values are from the three central Bay stations or Wooley Reef, nearly all are in surface waters, only occurred in spring and summer and show a similar pattern in the absolute and relative values of the size classes.

High biomass

Werribee and Williamstown represent the top five chlorophyll concentrations (>4.5 $mg.m^{-3}$) encountered during the year. Of the next five occurrences, three were at Sandringham (Station 3) and one each at Werribee and Clifton Springs (Station 6). When samples from all depths are considered, these four stations are the only ones which have chlorophyll concentrations above 3 $mg.m^{-3}$ (Table 3).

Werribee exhibited the highest values of standing stock of any station in September 1994. The micro fraction dominated the population contributing 75, 70 and 64% of the biomass to the surface, middle and bottom waters respectively, which represents chlorophyll *a* concentrations of between 5 and 9 $mg.m^{-3}$. Although the micro fraction was dominant, the other two size fractions also have high standing stocks (at least 1 $mg.m^{-3}$) during this event at all depths. The low F/Chl ratios found at all depths indicated that a high proportion of the chlorophyll was associated with either senescent or dead planktonic material. Whether this material was a remnant localised phytoplankton bloom or originated from recently resuspended benthic material is impossible to determine.

The pattern at Werribee in September 1994 of a high micro concentration representing greater than half of the biomass is apparent in only three of the other ten high values. In over half of these samples the micro fraction is under represented at 3-25% of total biomass. The nano fraction is the dominant size fraction in an equal number of samples as the micro, generally having concentrations of 2-4 $mg.m^{-3}$and representing 50-70% of the biomass. Williamstown shows this consistently. At this station the micro and pico fractions represent between a quarter and half of the standing stock of the nano fraction respectively in the majority of cases.

There are two cases when the pico fraction represents the dominant size class and total chlorophyll *a* remains higher than 3.6 $mg.m^{-3}$. Both of these occurred at Werribee and are surface values, nano values remained high on these occasions but the micro fraction was less than a tenth of the combined concentration of the other two fractions.

Low biomass

The three central stations and Wooley Reef (2, 8, 9 & 11) had a total chlorophyll concentration, at some depth, of less than 0.47 $mg.m^{-3}$, on between two and four sampling trips each (Table 5). Low total chlorophyll values were recorded in October and December 1994 at three of these four stations. All ten of these lowest chlorophyll values were recorded in Spring and Summer (September 1994 to January 1995).

The relative contribution of the three size fractions, when total chlorophyll is low, and the range of concentrations of chlorophyll values of the fractions, are similar across the sampling dates and stations. The pico fraction is the most conservative in terms of biomass, maintaining a concentration of at least 0.19 $mg.m^{-3}$, and contributing between 42% and 60% of the standing stock. The nano fraction does not exceed the pico concentration but was always greater than 0.13 $mg.m^{-3}$. This fraction is always at least 39% of the biomass under these conditions. In contrast to the other fractions, the micro sized organisms never represent more than 20% of the standing stock and are undetectable in over half of these samples. All four stations show at least one example of an occasion when there was no detectable micro biomass.

An observation of undetectable chlorophyll for a size class, from March 1994 to February 1995, did not happen in the pico fraction, occurred once in the nano fraction but occurred in one in ten samples analysed of the micro fraction (Table 6). The frequency of occurrence of undetectable chlorophyll in the micro fraction varies considerably with station, depth and season. On a per station basis it ranged from zero to eleven occurrences over the year at Blairgowrie and the Central Middle stations (Stations 1 & 11) respectively. Surface waters were much more likely to have no micro fraction present, particularly in the central and south-eastern end of PPB (Stations 2, 8, 9 & 11). Sandringham and Werribee showed regular depletion (12 and 21% of the time respectively) but are more likely to have no micro fraction in the middle or bottom water than the surface. Occurrences of "no micro fraction" are seasonal with 44% of occurrences in spring and 5% in winter (Table 7). This seasonal pattern is strongest in bottom water samples but is reflected in the other two depths.

Regulation of phytoplankton biomass in the water column is impacted by a number of physical and biological factors. Phytoplankton growth rates in PPB are estimated to be on average 365 mg of carbon fixed m^{-2} day^{-1}. For phytoplankton biomass to remain stable over annual periods this production must be lost from the water column either through maintenance respiration, grazing or sinking. The picoplankton and a proportion of the nanoplankton production are thought to be tightly coupled to heterotrophic microplankton grazing, leading to efficient recycling of nutrients and relatively stable biomass concentrations within the water column (Harris, 1986). The microplankton in contrast are considered to have lower affinities for nutrient uptake and are only able to compete when relative nutrient concentrations or fluxes are high. Since the larger grazers of these cells must respond to ephemeral increases in biomass there is usually a lag or temporal uncoupling between increased production and increased grazing. If physical mixing rates are low at these times then short term accumulations of biomass or 'blooms' of these algae can occur over short time periods (weeks). Nutrient or light limitation, along with physical mixing, are the most likely factors limiting the amount of biomass accumulation.

Sinking rates of diatom species have been postulated to be integral to their ecology. Many species are able to regulate their buoyancy, being able to sink to higher nutrient waters when growth is limited. In addition they are also able to increase the rate of descent by aggregating with each other and with marine detrital material through the production of extracellular polycarbohydrates. As the rate of aggregation is dependent on chance collisions, the higher the concentration of cells reached, the higher the rate of clearance of the cells from the water column. Calculations based on cell 'stickiness' have determined the maximal or critical concentration that certain species can attain, under certain physical conditions, before aggregation and sinking outpace growth (Tiselius & Kuylenstierna, 1996). Not all cells leaving the water column die or are consumed in the sediment. If buried and in cool conditions many form resting spores which can remain viable for months. Subsequent warm temperatures and resuspension into the water column can promote germination and maximal growth rates within days (McQuoid & Hobson, 1995). Counter to the theory that grazing accelerates the rate of flux of nutrients from the water column to the sediments through sinking fecal pellets (Hansen et al., 1996), it has been suggested that grazing pressure can reduce the probability of microplankton reaching their critical sinking concentration and so may prolong the time that nutrients are held in the water column.

Table 6: Stations listed by the number of occasions when chlorophyll *a* was undetectable in the micro fraction at three depths. (14 sampling trips)

Station	surface	middle	bottom	total	% total n=42
1	0	0	0	0	0%
4	0	0	1	1	2%
6	1	1	0	2	5%
8	2	0	1	3	7%
2	2	1	1	4	10%
3	1	2	2	5	12%
11	6	1	0	7	17%
5	2	3	4	9	21%
9	6	3	2	11	26%
Totals	**20**	**11**	**11**	**42**	**11%**

Table 7: Number of occasions, by season, when chlorophyll *a* was undetectable in the micro fraction.

Season	dates*	surface	middle	bottom	total	%
autumn	28/3-1/6/94	6	2	3	11	27
winter	26/6-16/8/94	0	2	0	2	5
spring	14/9-9/11/94	7	4	7	18	44
summer	6/12/94-1/2/95	7	3	0	10	24

* 3 field trips by nine stations

In contrast to the 1994/95 chlorophyll data the samples collected in the final five months of 1993 (August to December) show the regular occurrence of undetectable nano fraction in the eight stations sampled. Of the 112 samples collected an average of one in eight samples showed no detectable nano fraction, these comprised of 7 surface, 2 middle and 5 bottom values. In comparison over the same period in 1994 the lowest nano value recorded at the same stations was 0.16 $mg.m^{-3}$.

3.4. Vertical distribution of phytoplankton biomass through the water column

Most of the previous studies of chlorophyll *a* in PPB have used either surface, or integrated water samples from a number of discrete depths. Being a relatively shallow basin of 24m maximum depth with a fetch of greater than 50 km in all directions, PPB is considered to be vertically well mixed with little likelihood of persistent surface or sub-surface maxima in chlorophyll *a* concentration.

This study obtained extracted chlorophyll *a* samples from surface (0.5 metre), middle (half total depth) and bottom waters (1 to 2 metres above bottom) at each station sampled. Significant variation of chlorophyll *a* with total depth is common. Over the annual period March 1994 to February 1995 more than thirty percent of stations showed a difference between surface and bottom of more than 0.5 $mg.m^{-3}$ with over a third of these being differences of greater than 1.0 $mg.m^{-3}$.

All stations showed a difference, with depth, of at least 0.4 $mg.m^{-3}$ chlorophyll *a* at some time during the year. On average, when bottom waters are compared to surface water at each station, a pattern of change is apparent with the central stations having predominantly more chlorophyll on the bottom and the coastal stations more on the surface (Table 8).

Table 8: Statistics on annual depth difference in chlorophyll *a* , by station, March 1994 to February 1995.

Station (No.)	depth (m)	Mean difference * ($mg.m^{-3}$)	Mean Surface ($mg.m^{-3}$)	Average % difference Surf to Bot	median difference ($mg.m^{-3}$)	range* ($mg.m^{-3}$)
Mid Central (11)	21	0.31	0.76	41%	0.19	-0.38-1.62
Mid Nthn. (9)	22	0.28	0.86	32%	0.12	-0.56-1.59
Wooley Reef (2)	12	0.21	1.08	20%	0.20	-0.51-0.42
Mid Sthn. (8)	22	0.15	0.99	15%	0.17	-0.09-0.40
Blairgowrie (1)	17	0.11	1.02	10%	0.08	-0.11-0.51
Clifton Spring (6)	10	0.12	1.76	7%	0.08	-0.45-0.85
Sandringham (3)	10	-0.09	1.74	-5%	-0.05	-2.77-1.53
Williamstown (4)	11	-0.41	2.52	-16%	-0.26	-3.68-0.79
Werribee (5)	8	-0.84	2.80	-30%	-0.47	-3.37-0.45
Average	15					-3.37-1.62

* Values calculated as Bottom minus Surface concentration, therefore negative values indicate a surface accumulation of chlorophyll.

The magnitude of change with depth varies considerably between stations and the range of values is shown in Table 8. Werribee, Williamstown and Sandringham (Stations 5,4 & 3) show the greatest range of values, with all three stations exceeding the range of the rest by at least a factor of two. This large range is symptomatic of very high surface concentrations on a number of occasions at these three stations. The mean value for surface chlorophyll at these stations would significantly overestimate the bottom concentration. The Werribee station in particular was highly prone to this phenomenon, where bottom concentration was on average 30% less than that at the surface.

Interestingly the station at Clifton Springs (6), although classified as a central station on the basis of average standing stock, shows characteristics from this analysis that are intermediate between the coastal and central groups. This station had almost even

numbers of occurrences of negative and positive differences, and only just had a net positive value overall.

Comparison of the relative occurrence of differences in chlorophyll *a* with depth of the central and coastal stations are presented as quantile plots in Figure 13a. Logically, stations with higher average standing stock may be expected to have the potential for larger differences in chlorophyll concentration with depth. Indeed the coastal stations show the propensity for significant depth dependent changes in chlorophyll, with over 40% of stations measured showing greater than 0.5 $mg.m^{-3}$ difference, and about 10% showing differences of greater than 2 $mg.m^{-3}$. This group of stations has chlorophyll surface values higher than bottom values in over 60% of samples.

Stations in the central group (1,2,8,9&11) did not ever have a surface value that was more than 0.56 $mg.m^{-3}$ greater than the bottom value. The central stations have only 20% of values more than 0.5 $mg.m^{-3}$ and only 5% greater than 1 $mg.m^{-3}$ over the full depth at a station. Bottom values are in excess of surface values more than 75% of the time, leading to an average increase in chlorophyll with total depth (Figure 13a). The Central Mid, and Central Nth Stations (11 & 9), are the most striking examples of this type of station. Although mean surface chlorophyll at these stations is only 0.76 and 0.86 $mg.m^{-3}$ respectively, the average increase in chlorophyll with total water column depth is 0.31 and 0.28 $mg.m^{-3}$, representing an average increase with depth of 41% and 32% for the year (Table 8).

There is a strong seasonal trend in the difference of chlorophyll with depth at both the central and coastal stations over the period March 1994 to February 1995. Seasonal trends in chlorophyll concentration for all three depths and the three size fraction are described in a previous section (Figure 9b). Differences in seasonal average surface or bottom total chlorophyll values follow different trends in the two groups of stations (Figure 13b). Overall, coastal stations show average surface chlorophyll values greater than bottom values in all seasons except winter where there is a slight bottom excess. The central stations have an excess of chlorophyll in bottom waters over all seasons. Seasonal trends for chlorophyll values at these two depths may be highly correlated. Both sectors show a significant decrease in chlorophyll between winter and spring and central stations show a general increase in chlorophyll between autumn and winter. In contrast, changes in chlorophyll between seasons may be negatively correlated (autumn to winter and spring to summer, coastal group) or weakly correlated (spring to summer, central group).

Both sectors of the Bay have very little average difference with depth during winter and spring. At the central stations, significant accumulations of chlorophyll occur in bottom waters during summer, leading to an increase of 35% with depth. Autumn and winter differences are ameliorated by increases in bottom water concentration at least as great as changes in surface waters. The relative increase in chlorophyll in bottom waters from autumn to summer accounts for the majority of change in the overall water column chlorophyll content between these seasons. In contrast, coastal stations show a decrease in chlorophyll in bottom waters from spring to summer with only a slight increase in surface waters. The major change in concentration occurs between summer and autumn when surface water concentrations increases by 80%, this then dissipates over winter and spring. Bottom water again lags, reaching maximal concentrations in winter (Figure 13b).

The three size fractions all contribute to the average seasonal depth differences in both sectors (Figure 14). Relative differences between size fractions in particular seasons can be both positively (autumn and summer) or negatively correlated (winter and spring). The pico fraction is the most conservative in terms of depth differences and generally follows predictable patterns of change at both depths. The nano fraction has a high level

of depth difference in the coastal sector (particularly autumn) but is the most conservative in the central sector. The micro fraction has a relatively consistent depth difference over both sectors and all seasons except spring. The higher micro fraction bottom chlorophyll values during winter in both sectors effectively balance surface accumulations in the other fractions leading to appearance of apparently homogeneous total chlorophyll values (Figure 13b).

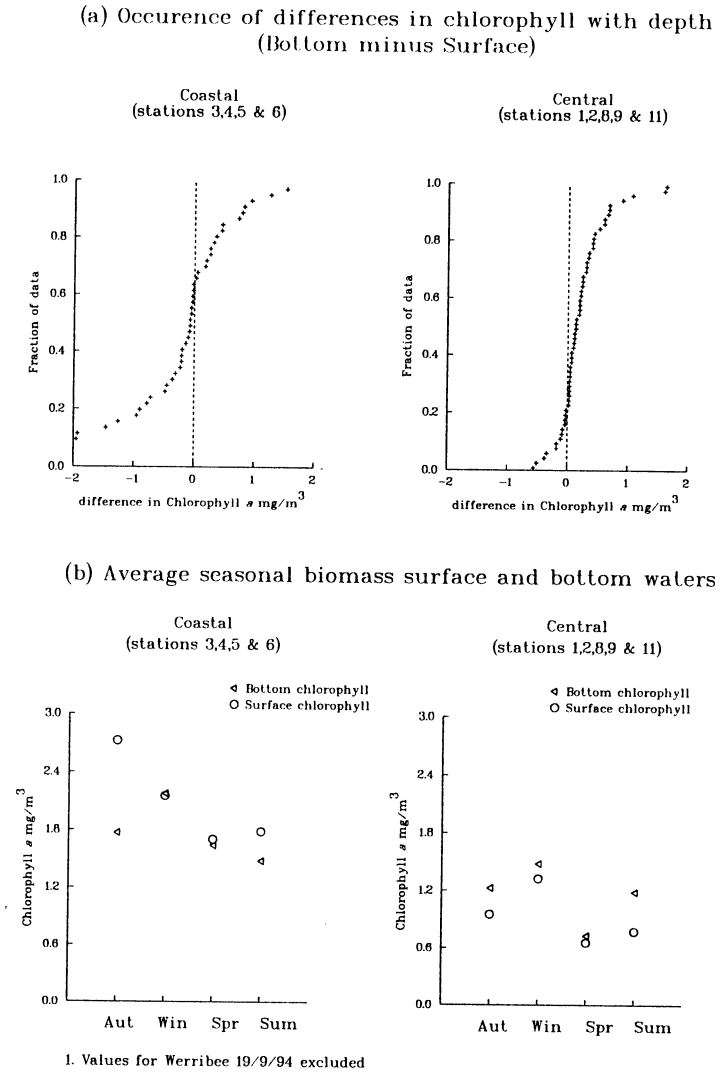


Figure 13: [A] Quantile plots of differences in chlorophyll concentration between surface and bottom waters for "high" or "low" chlorophyll concentrations i.e central or coastal stations [B] Seasonal variation in chlorophyll concentration in bottom or surface waters for "high" or "low" chlorophyll stations

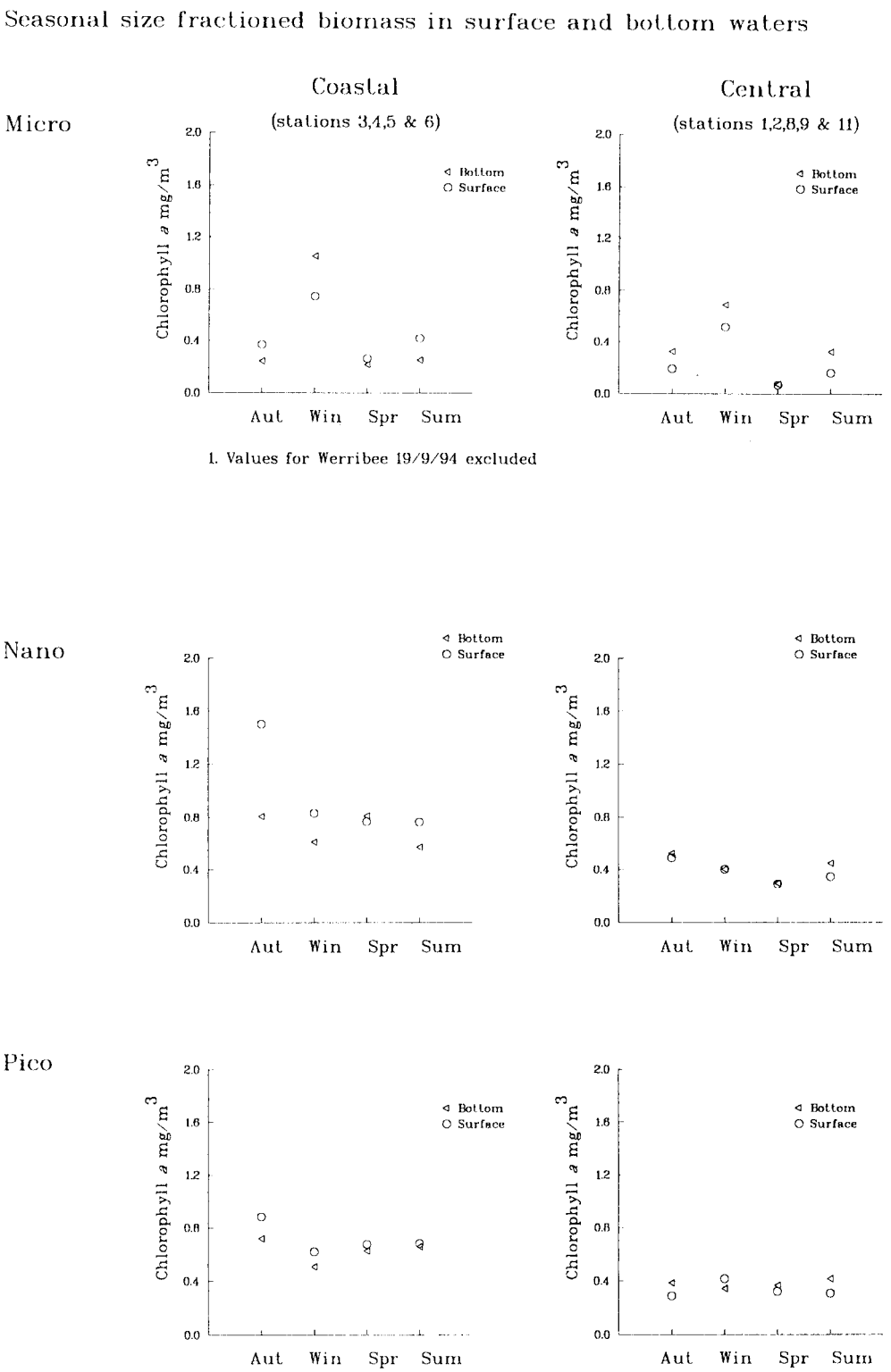


Figure 14: Seasonal variation in chlorophyll *a* concentration in bottom or surface waters for "high" or "low" chlorophyll stations (i.e central or coastal stations) in micro (upper), nano (middle) or pico (bottom) fractions.

3.5.Water column integrated phytoplankton biomass (chlorophyll *a* . m^{-2})

In ecological studies, determining the concentration of phytoplankton on an areal basis is the first step in estimating the absolute standing stock in an area or system. Often this estimate can also be used in discerning system processes that may be enhancing or limiting phytoplankton production, in particular the effect of light.

3.5.1. Station statistics

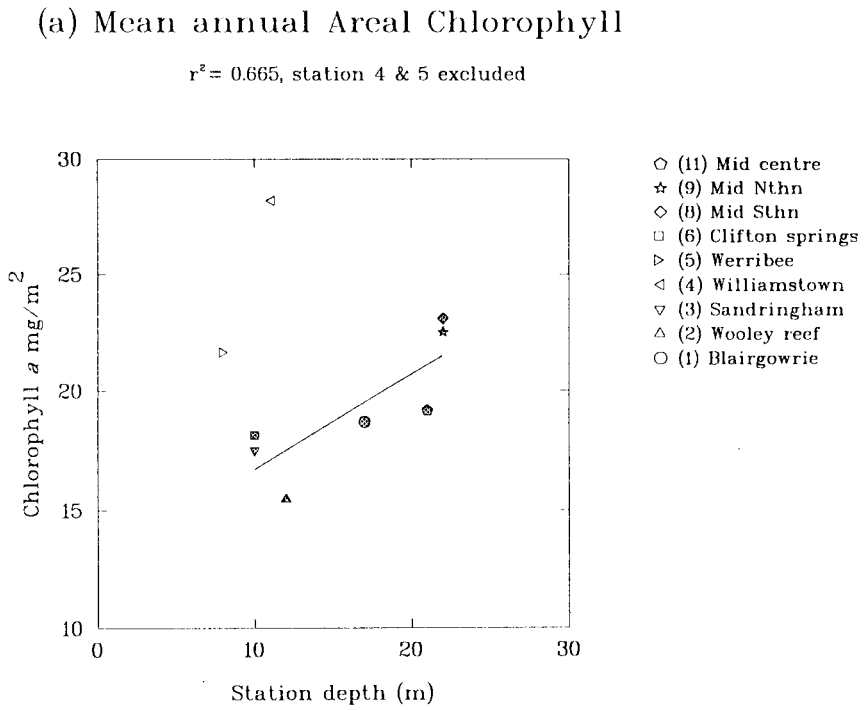
Results for annual station chlorophyll *a* under one square metre, calculated as the average value of the surface, middle and bottom concentrations multiplied by the depth in metres are presented in Table 9. The most striking feature of the mean values when compared to mean concentration values per cubic metre (Table 3), is the much smaller range of values, less than a two fold difference. In addition the stations do not fall within the previously described coastal and central sector groupings.

Table 9: Annual Total Chlorophyll *a* Station Statistics, March 1994 to February 1995.

Station (No.)	depth (m)	Mean (mg/m^2)	standard deviation	median (mg/m^2)	range (mg/m^2)
Williamstown (4)	11	28.18	10.13	24.75	8.80-45.00
Mid Sthn. (8)	22	23.10	11.37	19.74	10.71-49.56
Mid Nthn. (9)	22	22.53	11.43	20.46	11.22-51.48
Werribee (5)	8	21.65	21.71	11.84	7.36-85.76
Mid Central (11)	21	19.17	9.17	18.69	8.61-42.84
Blairgowrie (1)	17	18.69	6.46	16.15	11.90-33.32
Clifton Spring (6)	10	18.14	8.58	12.90	8.40-36.70
Sandringham (3)	10	17.52	9.17	14.90	8.40-35.90
Wooley Reef (2)	12	15.46	5.48	15.34	8.19-26.26
Average	15				

3.5.2. Difference with depth

As noted previously, the classification of the high chlorophyll concentration (coastal) and low chlorophyll concentration (central) station groupings also conveniently divided the stations on sample depth differences. As the calculation of chlorophyll per metre ^{2} will remove any such sample depth related factors, the calculated values for compared station to station could indicate possible station depth dependant trends. Both mean and median values show a general trend of increasing chlorophyll per square metre with increasing station depth (Figure 15 a,b). When mean values are considered, if Williamstown and Werribee are not included, a just significant ($p < 0.05$) correlation of increasing chlorophyll with depth is apparent. Median values are significantly ($p<0.001$) and highly correlated with depth, when Williamstown is excluded from the analysis.



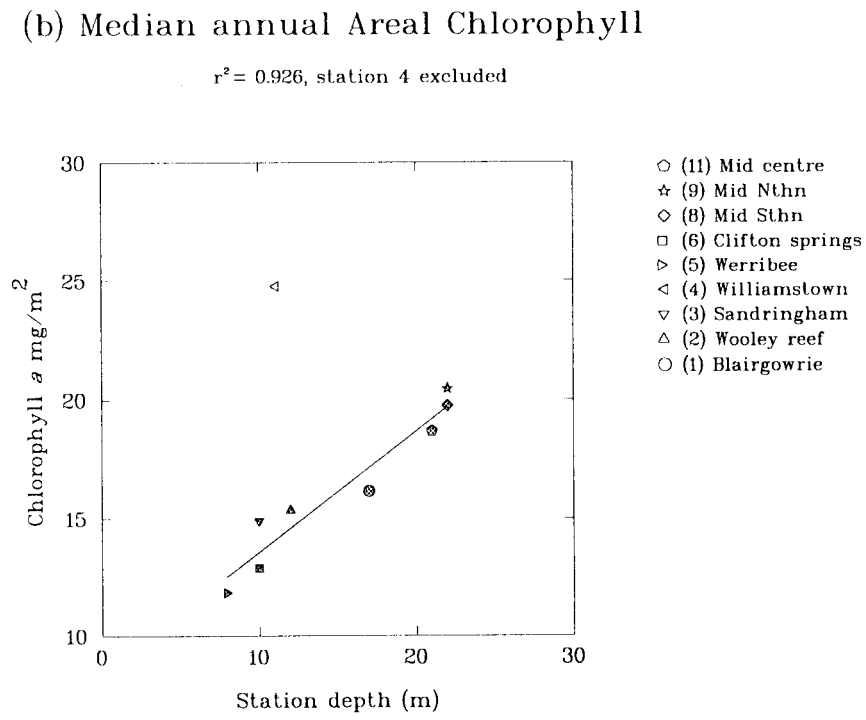


Figure 15: Mean (upper graph) and median (lower graph) integrated water column chlorophyll *a* values as a function of station depth.

3.5.3. Seasonal Changes

Calculation of seasonal trends in chlorophyll m^{-2} for any one station will logically be the same as the three depth averaged results for chlorophyll m^{-3}. The relative distribution of chlorophyll m^{-2} has been shown to be significantly correlated with depth over an annual period. Figure 16 presents the relationship of areal chlorophyll to depth for six stations and each season between March 1994 and February 1995. The values for the Williamstown station are not presented as this station has consistently high values.

Reasonable correlations of areal chlorophyll with depth are apparent in summer (r^2 = 0.334, $p<0.05$) and, if the Werribee station is excluded, autumn (r^2 = 0.339, $p<0.05$). A weak relationship persists in winter which has completely disappeared by spring. The change in areal chlorophyll with station depth during summer and autumn is largely contributed by the nano phytoplankton (r^2 = 0.495 and 0.335 respectively) with a lesser contribution from the pico phytoplankton (r^2 = 0.249 and 0.193 respectively). During spring the areal chlorophyll values are very consistent across the whole Bay, except for Williamstown. This period corresponds to an extremely low microphytoplankton biomass and hence a large percentage contribution to total chlorophyll by the two smaller size fractions.

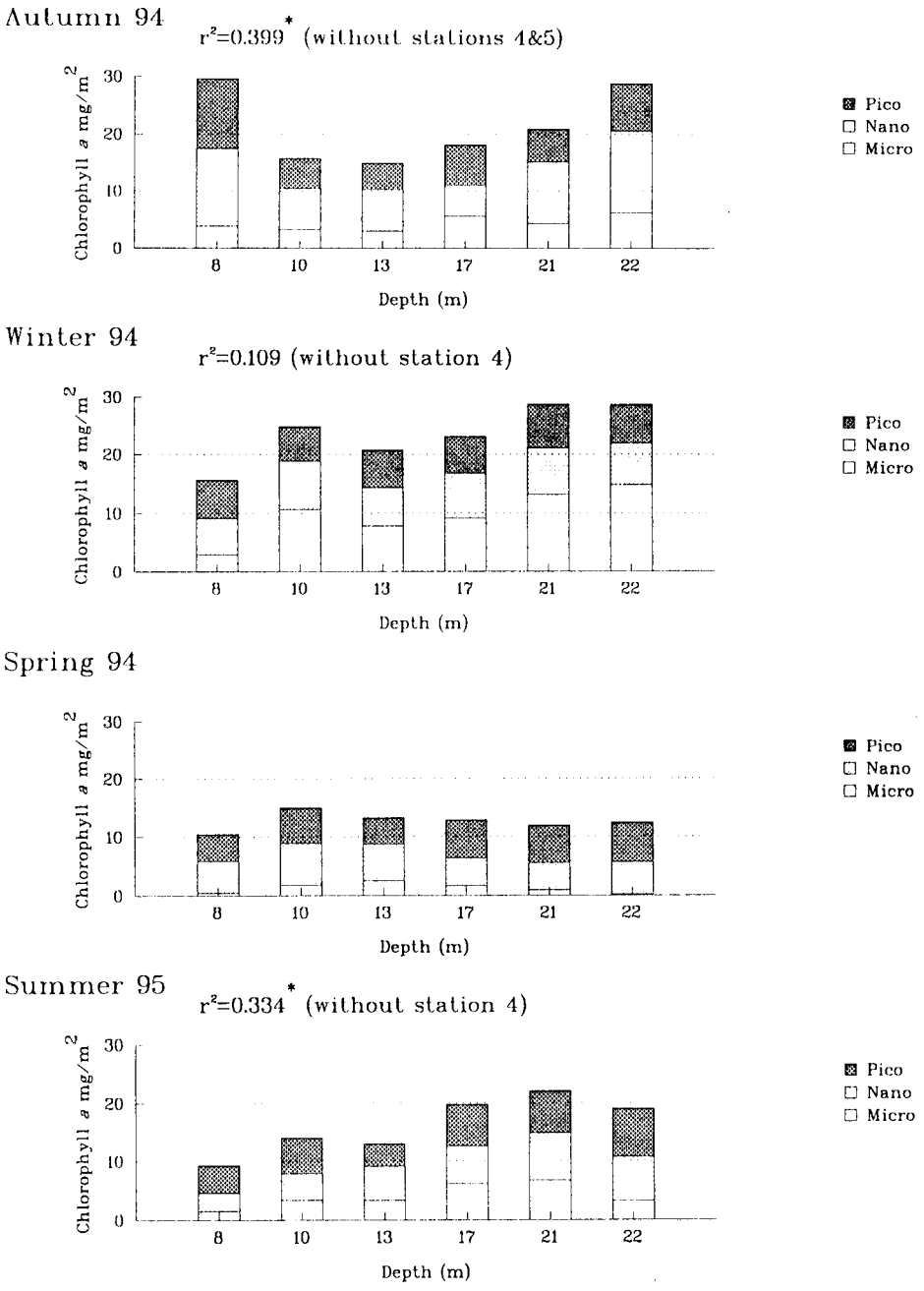


Figure 16: Bar graphs showing the contribution of each size fraction to the overall water column integrated chlorophyll *a* content as a function of station depth. Data are given for each season.

3.6. Statistical analysis of chlorophyll distributions

3.6.1. Statistical analysis of the distribution of chlorophyll concentration

Observation of the chlorophyll per cubic meter concentration values has indicated that certain stations have similar characteristics and may fall into specific groups which would facilitate Bay-wide analysis. Inter-station parametric statistical analysis of the total and specific size fractions was attempted for the log transformed data. However the Bartlett test for non-homogeneity of variance was always highly significant. The non-parametric Kruskal-Wallis ranking test was run as an alternative method on the raw data. This test gives each of the values in the pooled data a rank score and in the first instance determines if the summed rank scores for stations is significantly different across all stations. Further pairwise tests can then be performed to resolve which stations are significantly different from each other. Results of Kruskal-Wallis tests for each of the size fractions and different combinations of the size fractions are presented in Figure17. Results in this analysis correspond to the average ranked score of each station with the lowest score and chlorophyll concentration on the left and the highest on the right. Stations which are not significantly different at the $p<0.05$ level are linked by a solid line. When all the fractions are considered together the stations are ranked in the same order as their mean chlorophyll concentrations (Table 3), except that Werribee (5) and Williamstown (4) exchange positions. A continuum of increasing chlorophyll is indicated with stations only being significantly different to stations ranked 4 or 5 places higher or lower in concentration. On the basis of total chlorophyll, the stations would be split into two significantly different groups with stations 11, 9, 8 and 1 being central and 4, 5 and 6 coastal. Sandringham (3) and Wooley Reef (2) are intermediate to these groups showing some similarity to both central and coastal groups. When the size fractions are considered, either individually or in combination, a similar pattern of coastal and central stations emerge. However the distinction between the groups increases with decreasing cell size and the two groups become separated completely when the two smallest fractions are combined. Here the distinction between the shallower northern and western stations (3, 4, 5 & 6) from the Southern and central stations (1, 2, 8, 9 & 11) is complete.

3.6.2. Statistical analysis of inter-station differences in water column chlorophyll (chl a/m^2)

Comparisons of stations was determined by Kruskal-Wallis tests (cf chl m^{-3}) on annual areal chlorophyll for each of the size fractions and the combination of all fractions (Figure 18). At the $p<0.05$ level only stations 5 and 4 (when total chlorophyll was considered) and stations 2 and 9 (when the pico-phytoplankton were considered) showed significant differences. Previously determined (on the basis of chlorophyll per cubic meter) groups of coastal and central chlorophyll stations were not apparent, with station rankings instead indicating that for the two larger fractions Williamstown had elevated chlorophyll levels but that the deeper stations were in fact on the whole relatively higher in chlorophyll than the more northerly and western stations (see “Differences with depth”, this section).

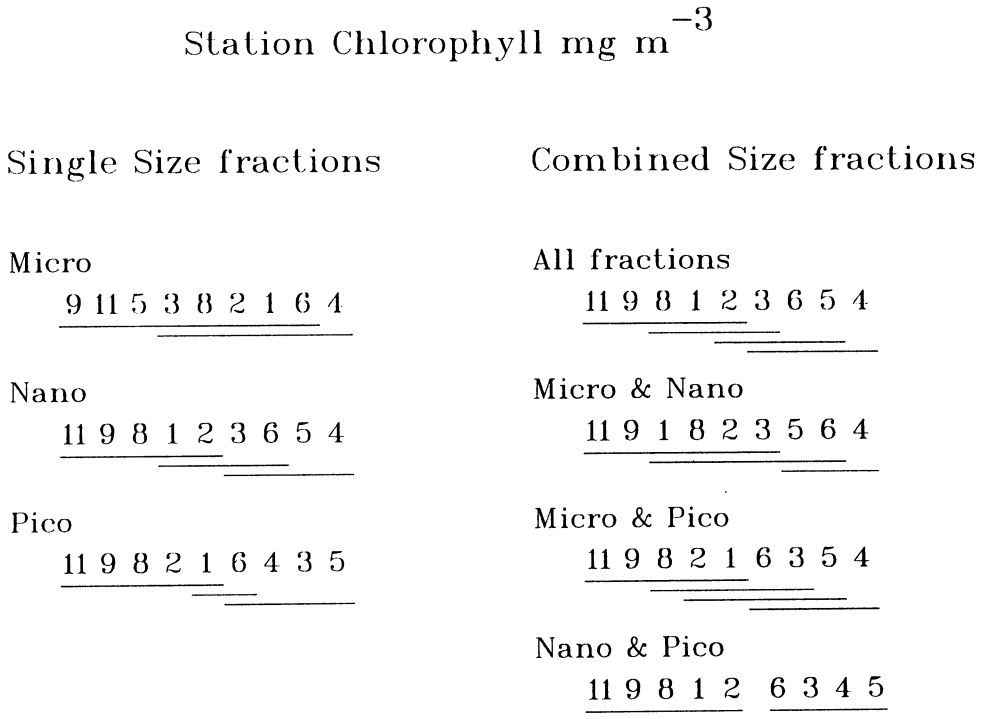
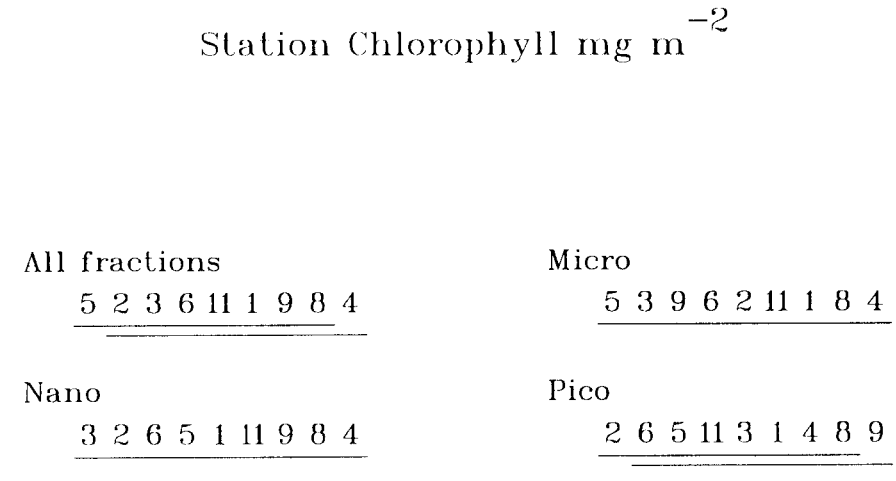


Figure 17: Results of Kruskal-Wallis tests on chlorophyll concentration distributions. Stations (numbers) with lowest score (chlorophyll concentration) appear on the left and those with the highest values on the right of a group. Stations which are not significantly different at the P<0.05 level are linked by a solid line. Results are given for individual size fractions and various combinations.



igure 18: Results of Kruskal-Wallis tests on chlorophyll areal distributions. Stations (numbers) with lowest score (chlorophyll concentration) appear on the left and those with the highest values on the right of a group. Stations which are not significantly different at the P<0.05 level are linked by a solid line. Results are given for individual size fractions and the combined total population

3.7. Primary productivity

3.7.1. Seasonal variation in photosynthetic capacity of the phytoplankton at the different stations.

The variation, by station, in biomass-corrected photosynthetic capacity (assimilation number) of phytoplankton during the study period is shown in Figures 19 - 22. For the total population (Figure 19) it is apparent that there was a major peak in Pmax during February/March 1994, after which values declined to a low over winter and tended to rise again in the following spring and summer. This seasonality was common to all stations, although the magnitude of the value of Pmax varied. However, when the individual size fractions were considered, such a clear pattern did not emerge.

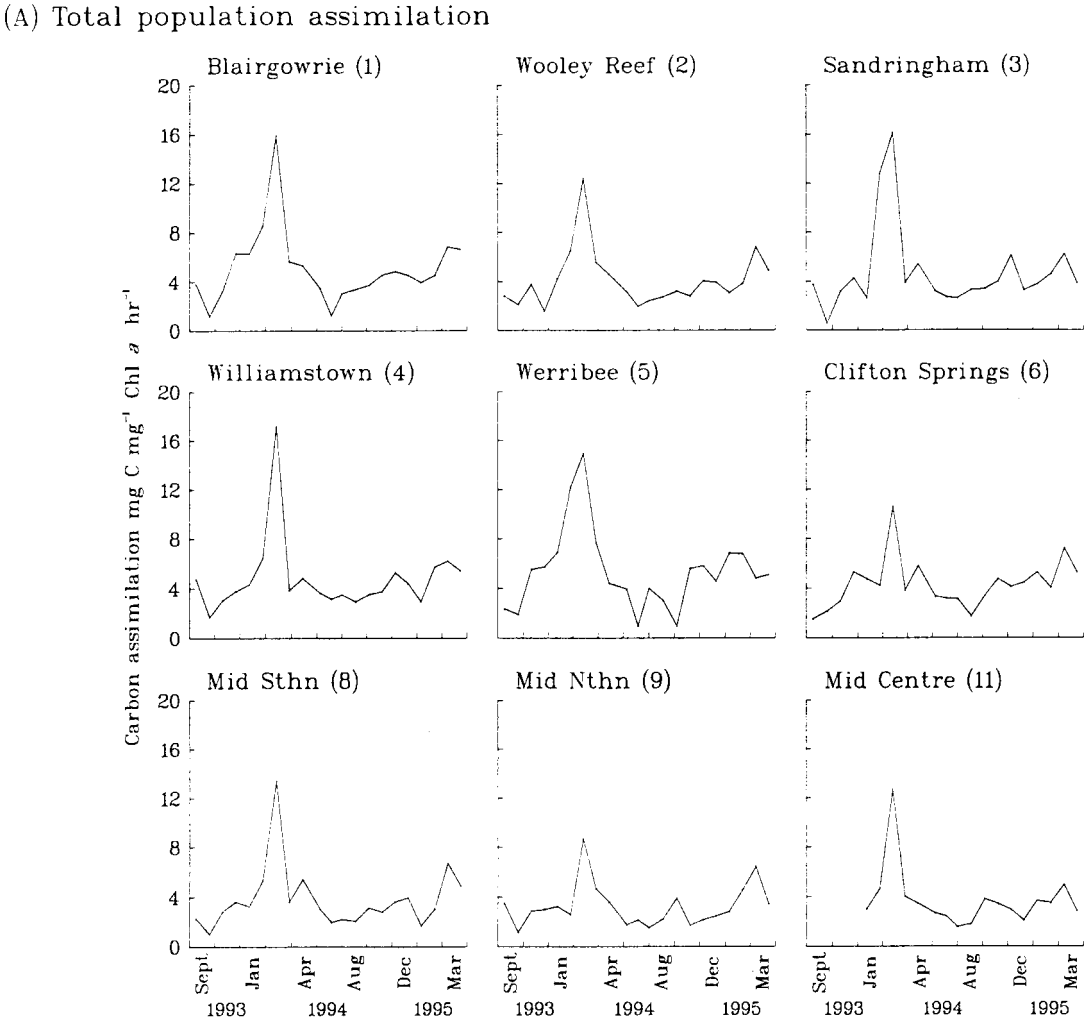


Figure 19: Seasonal variation in assimilation number of the whole phytoplankton population by station.

For the micro fraction (Figure 20), the magnitude and timing of the summer 1993/94 peak in Pmax was dependent on station, such that at Blairgowrie (station 1), a sharp peak was recorded in March 1994 whereas at Sandringham (station 3), the maximum was found in February / March 1995 and a noticeable peak was not found the previous summer. At other stations (e.g. Werribee, station 5), the period of high Pmax values extended over several months.

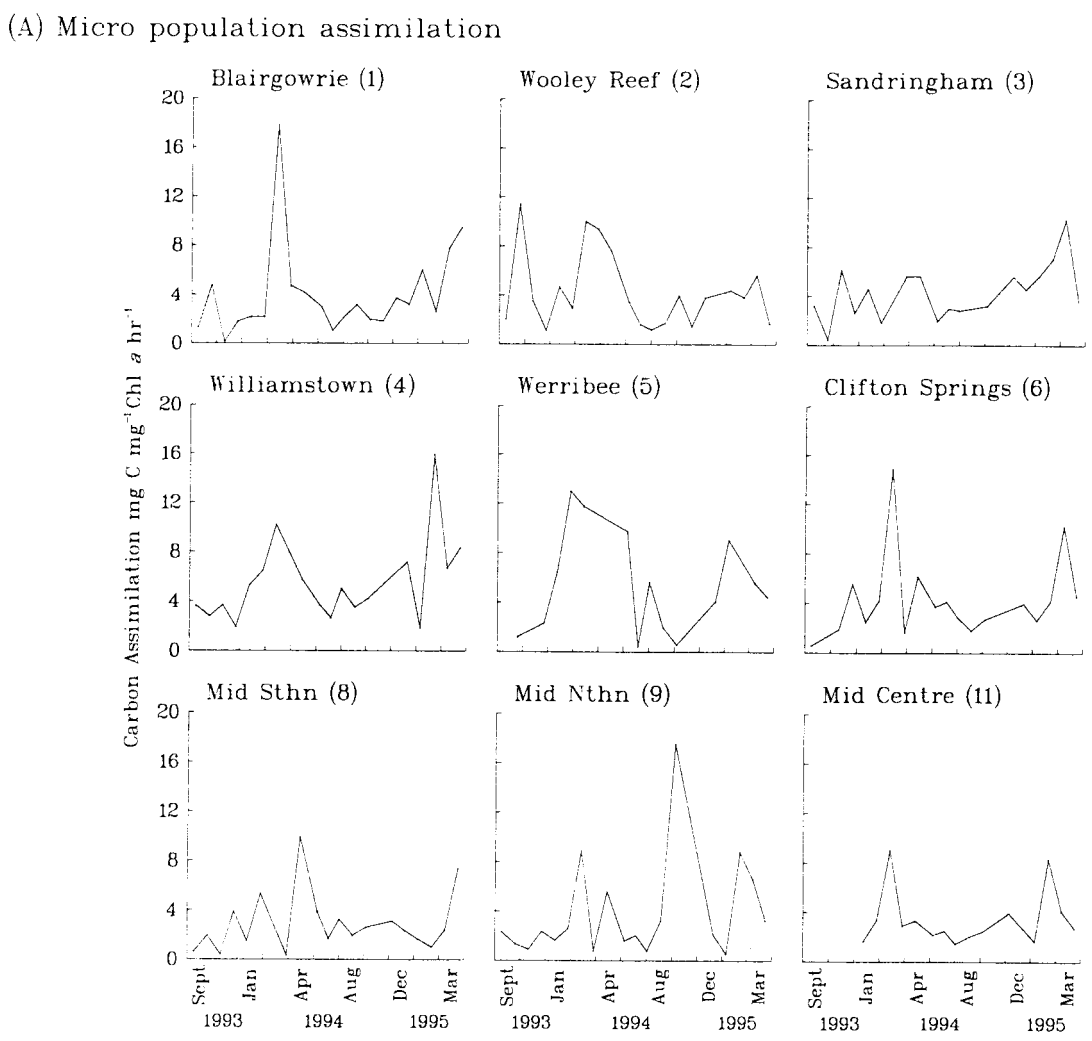


Figure 20: Seasonal variation in assimilation number of the micro fraction by station.

The nano fraction (Fig 21) showed multiple peaks in Pmax throughout the year at several stations (e.g. Blairgowrie, Sandringham), although for others (Werribee, Clifton Spring) the values of Pmax were dominated by a peak within the period December 1993 - March 1994.

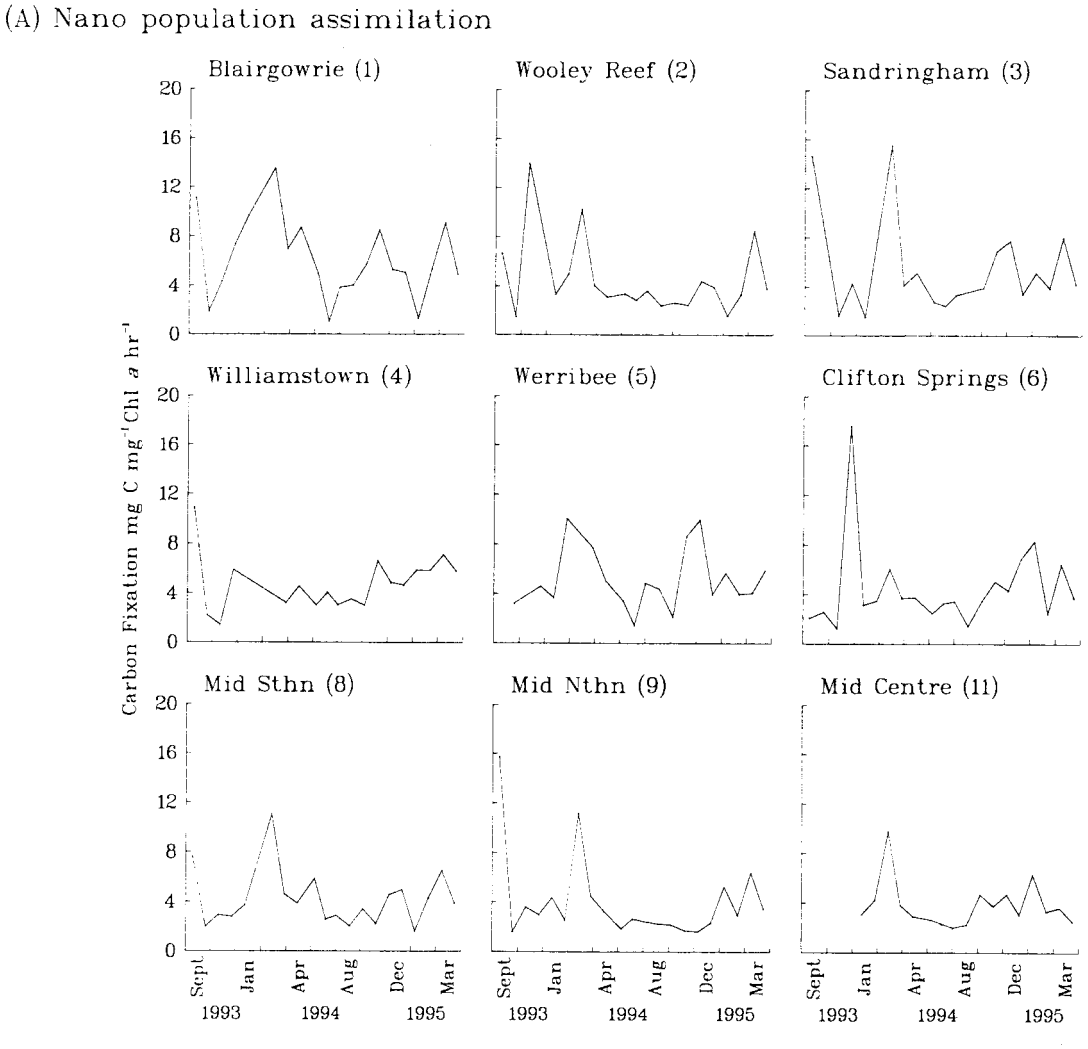


Figure 21: Seasonal variation in assimilation number of the nano fraction by station.

Multiple and / or broad peaks in Pmax were also a feature of the pico fraction (Figure 22). As for the other fractions, the different stations showed variations in timing and magnitude of maxima and minima in Pmax. In general terms, maximal values of Pmax were observed in late summer 1994. Following a decline through winter, Pmax rose to high values in summer 1995 though, as with the other fractions, the absolute values attained were not as high as in the previous year.

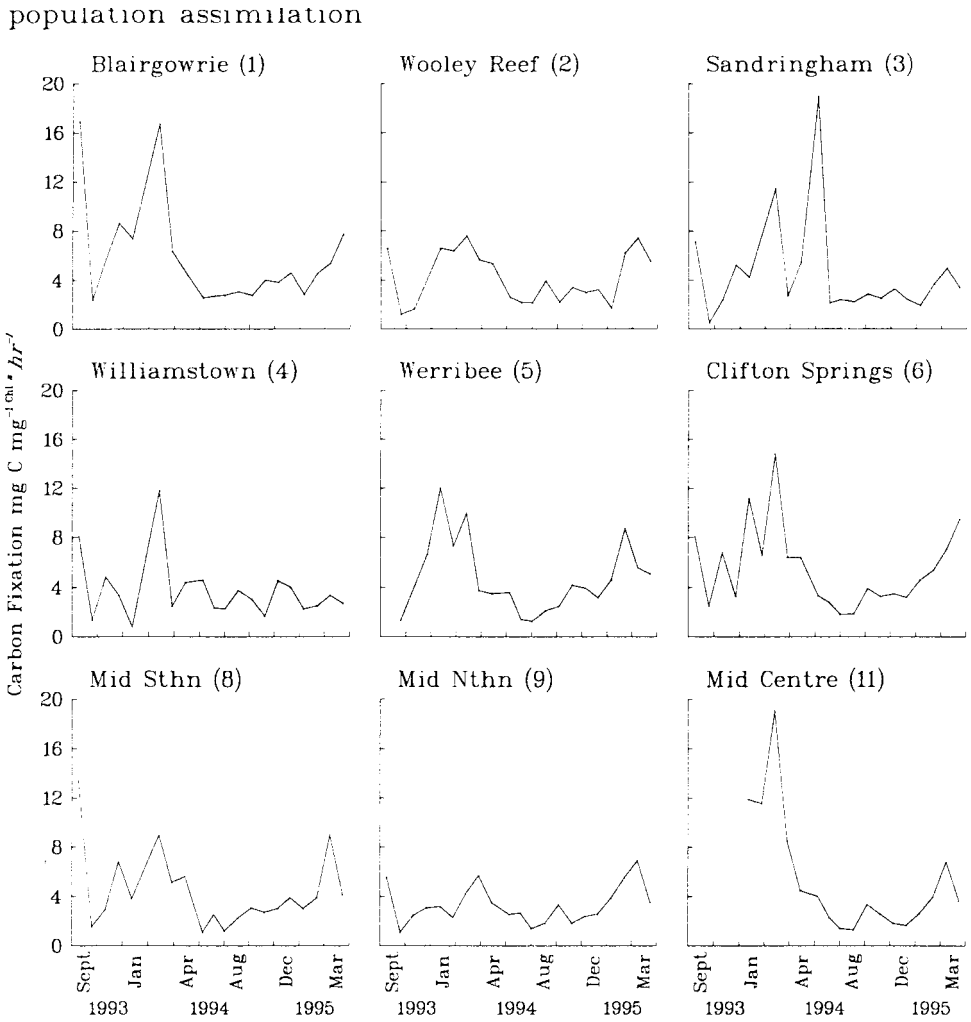


Figure 22: Seasonal variation in assimilation number of the pico fraction by station.

3.7.2. Seasonal changes in photosynthetic characteristics of the phytoplankton

The photosynthetic characteristics of the total population and individual size fractions were determined for a subset of the stations on each sampling occasion (see Table 1). The data obtained for each month are presented in Figures 23-26 for totals, micro-, nano- and pico-plankton respectively, where each data point represents values from one station. Figure 27 shows the smoothed (distance weighted least squares) trendline through all points for the data in Figures 23-26. In Figure 28 we present data for Ik for the pico fraction plotted with the Bay-wide average daily light penetrating to 2m during the previous 24 h, calculated from incoming irradiance and attenuation data for each station. No clear correlations were apparent with the Ik, or any other parameter, for other fractions so, for clarity, these have been omitted.

Clearly there was considerable variation in all parameters between stations. This variation was more marked in the micro fraction but occurred to a lesser extent in all fractions. The overall trends however indicate a maximum in Pmax during the summer of 1993/94 with values dropping to a minimum in winter of 1994 before rising slowly to high values in the late summer of 1995. Nano- and pico-plankton showed especially high values of Pmax during late summer 1994 resulting in a maximum for the total population that was greater than that achieved in the following year. However, the microphytoplankton showed the opposite trend with Pmax values higher in summer 1995 than in 1994. Furnas and Mitchell (1988) found in samples collected from surface waters and from around 100 m from the Coral Sea in October-November that maximum assimilation numbers for the < 2mm fraction were at least twice those recorded for the > 2 mm fraction. In a study covering a year, a number of time scales and two locations, Malone and Neale (1981) found that light saturated assimilation numbers for the > 22 mm and < 22 mm fractions reached similar maximum levels but that these maxima occurred at different times of the year. During the period up to autumn 1994, assimilation numbers for the nano and pico fractions in Port Phillip Bay were indeed higher than those of the micro fraction. However, after this time there were no significant differences in the values for this parameter among the three fractions.

Alpha values varied greatly and no seasonal trend was apparent. During the first half of the study, pico- and nano-plankton exhibited higher alpha values than the micro fraction reflecting the previously reported higher quantum efficiency in picoplankton (Morel & Bricaud 1981). This was not the case in spring 1994/summer 1995 when it was not possible to show any differences between photosynthetic efficiencies of the three fractions.

Similarly, the picoplankton showed lower light compensation points than the other fractions. This could again reflect the theoretically better efficiency of light harvesting in these organisms (Morel & Bricaud 1981) and their better performance at low light. Interestingly, the latter part of the study was characterised by slightly higher calculated average light intensities at 2m (Figure 28).

The photon flux at which photosynthesis becomes saturated is indicated by the parameter Ik (=Pmax/alpha). Low values of Ik suggest a population that is more shade adapted than one with high Ik. Ik in the micro and nano fractions varied throughout the study and no consistent seasonal pattern emerged.

However, the pico fraction showed a strong seasonal pattern with high Ik values recorded in summer and low values appearing in winter. This pattern bore a striking resemblance to the average daily photon flux at 2m for the preceding 24 h period (Fig 28). There was a similar but far weaker correlation for the nano fraction and values for the micro fraction were consistent throughout the year.

These data point to the pico fraction (and, to a lesser extent, the nanoplankton) being highly responsive to the prevailing light environment. It is possible that photosynthesis in these organisms is more likely to be regulated by light in Port Phillip Bay than that of the larger phytoplankton.

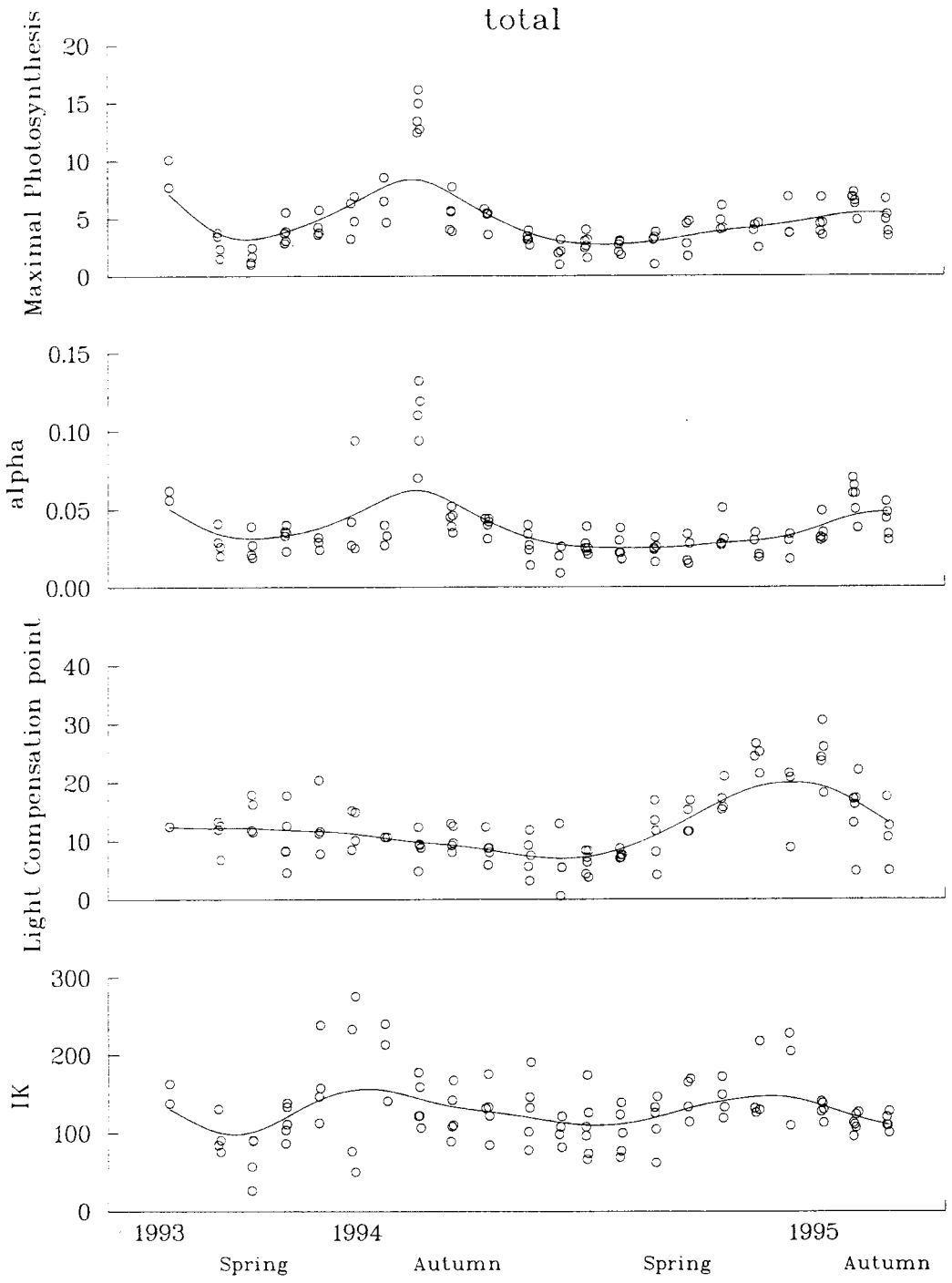


Figure 23: Seasonal variation in Bay-wide averaged values of maximal photosynthetic rate (assimilation number), alpha, light compensation point (LC) and Ik. Data are for the total phytoplankton population and each point represents data from a single station. The line is the distance weighted least squares smoothed value. Units are mgC. mg^{-1} chl *a*. h^{-1} (maximal photosynthesis); mgC. mg^{-1} chl *a*. $h^{-1}.(\mu Ein.m^{-2}.s^{-1})^{-1}$ (alpha) and $\mu Ein.m^{-2}.s^{-1}$ (Ik and light compensation point).

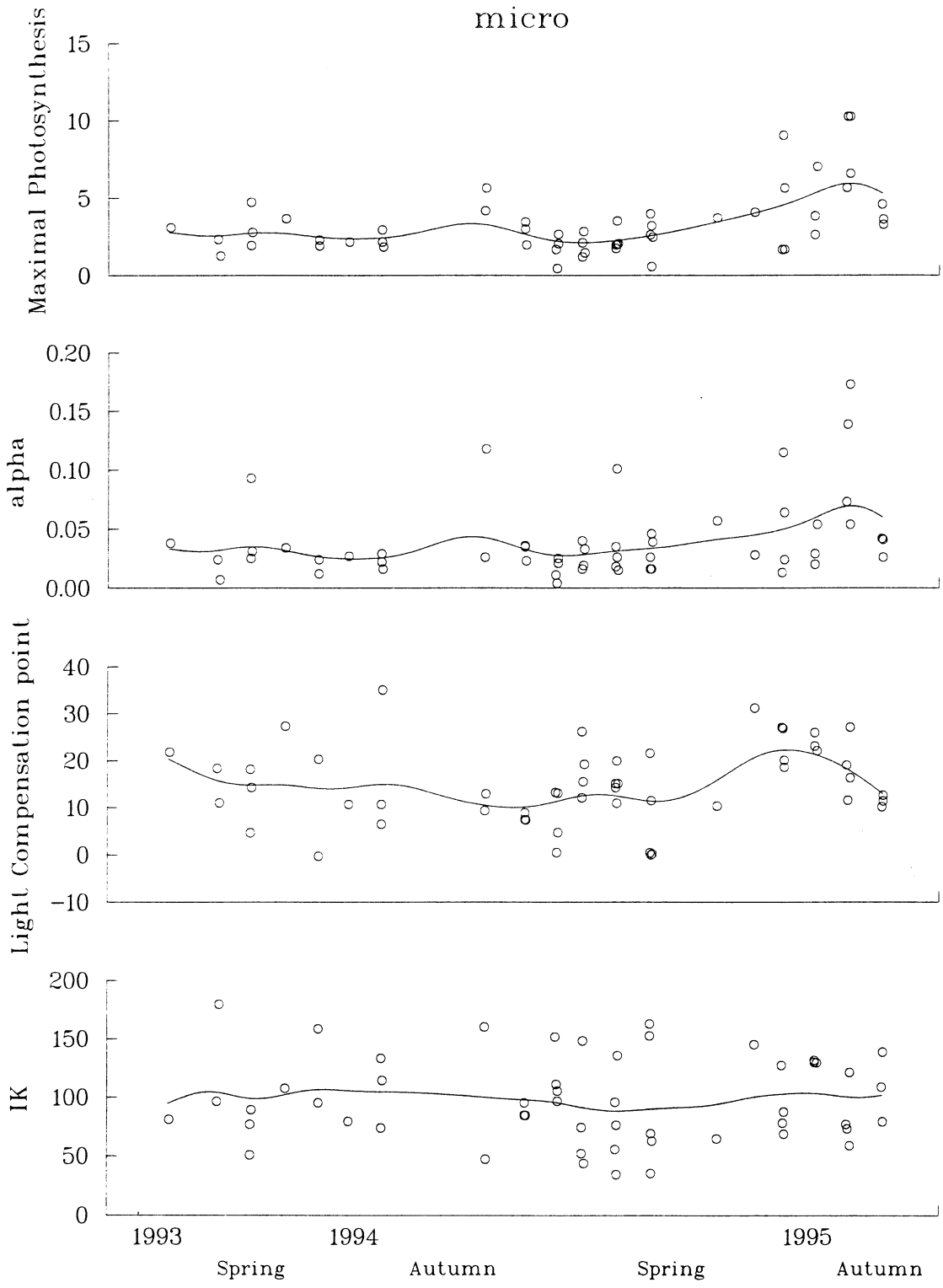


Figure 24: Seasonal variation in Bay-wide averaged values of maximal photosynthetic rate (assimilation number), alpha, light compensation point (LC) and Ik. Data are for the micro fraction and each point represents data from a single station. The line is the distance weighted least squares smoothed value. Units as for Figure 23.

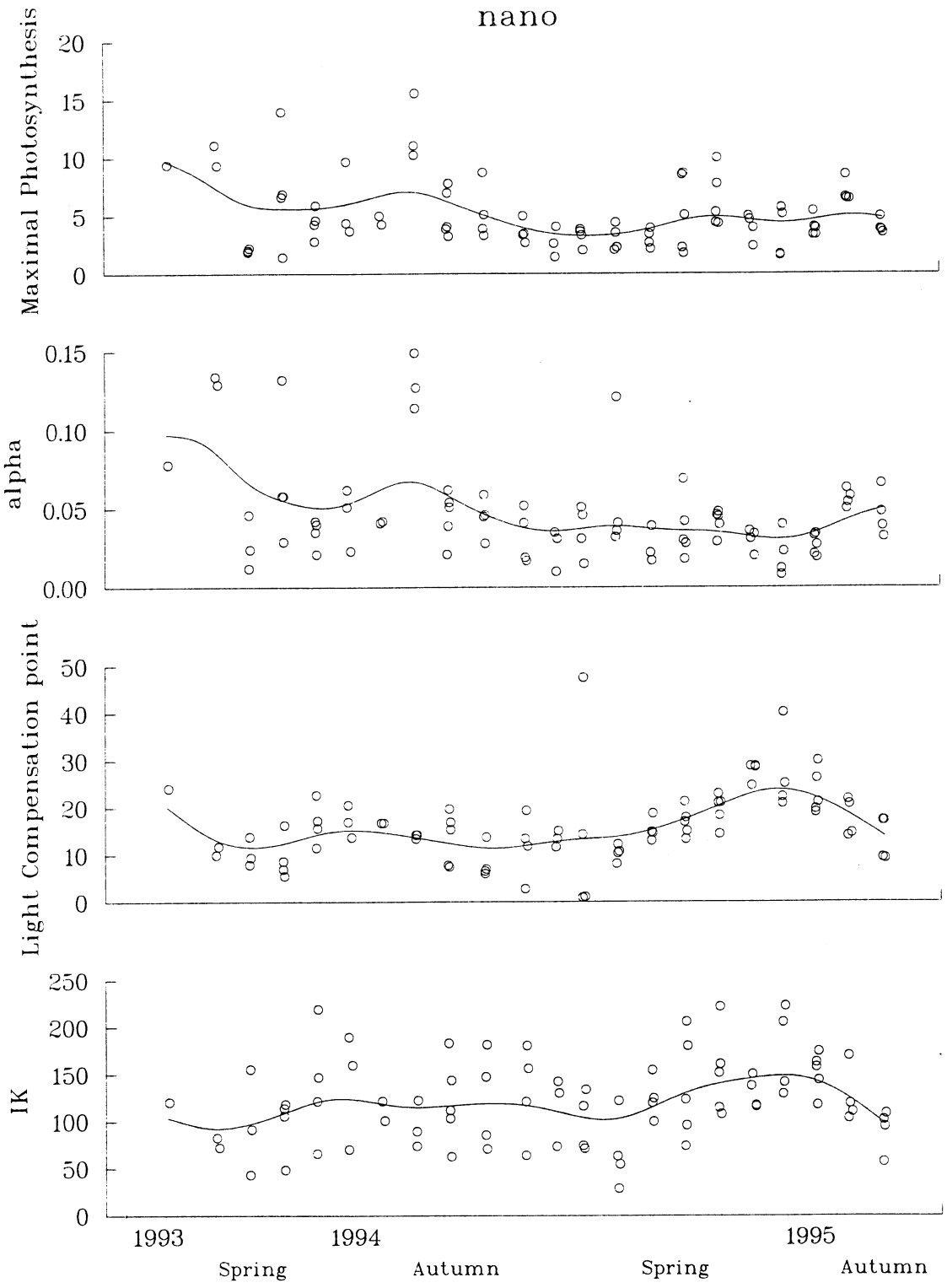


Figure 25: Seasonal variation in Bay-wide averaged values of maximal photosynthetic rate (assimilation number), alpha, light compensation point (LC) and Ik. Data are for the nano fraction and each point represents data from a single station. The line is the distance weighted least squares smoothed value. Units as for Figure 23.

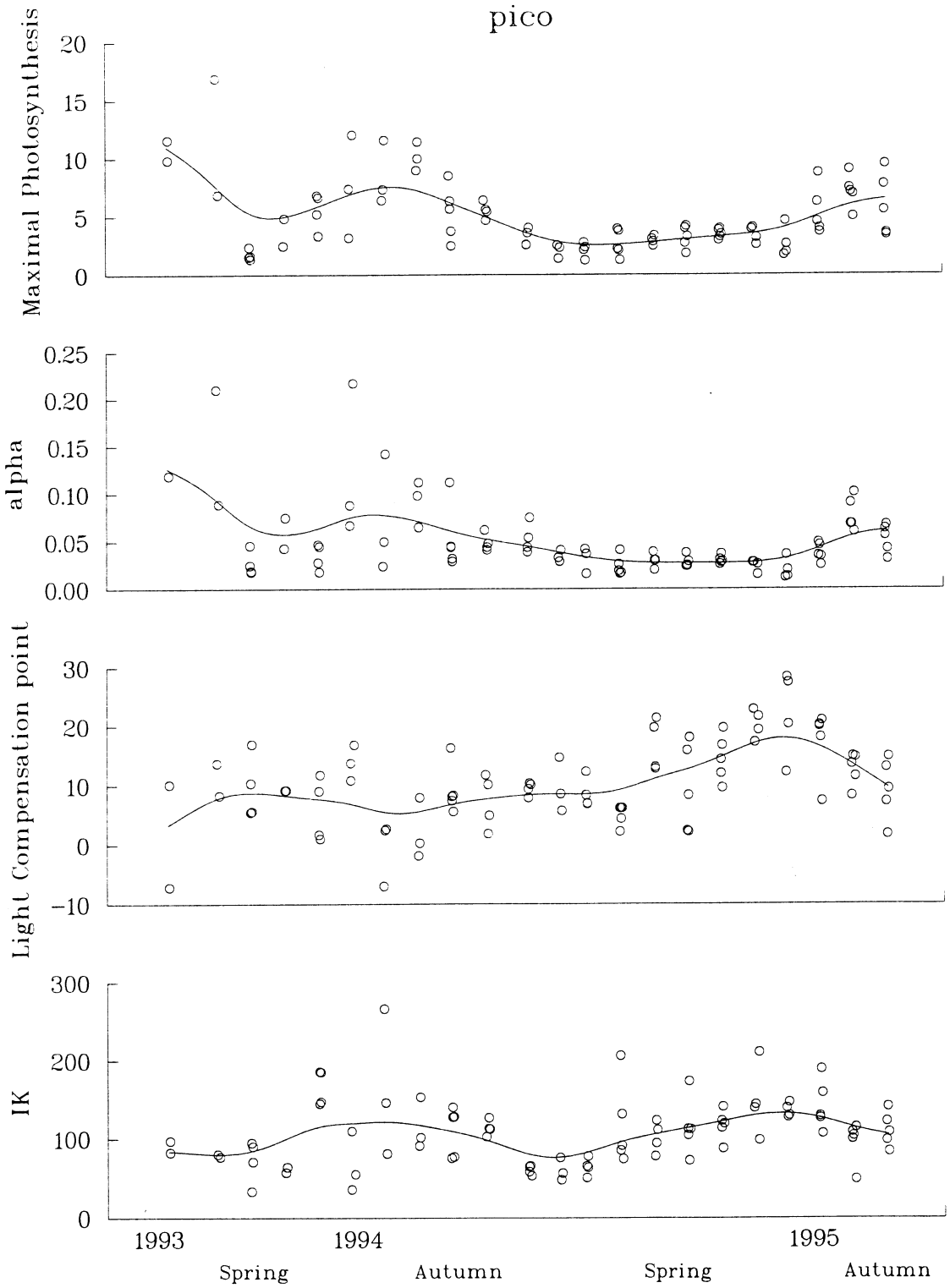


Figure 26: Seasonal variation in Bay-wide averaged values of maximal photosynthetic rate (assimilation number), alpha, light compensation point (LC) and Ik. Data are for the pico fraction and each point represents data from a single station. The line is the distance weighted least squares smoothed value. Units as for Figure 23.

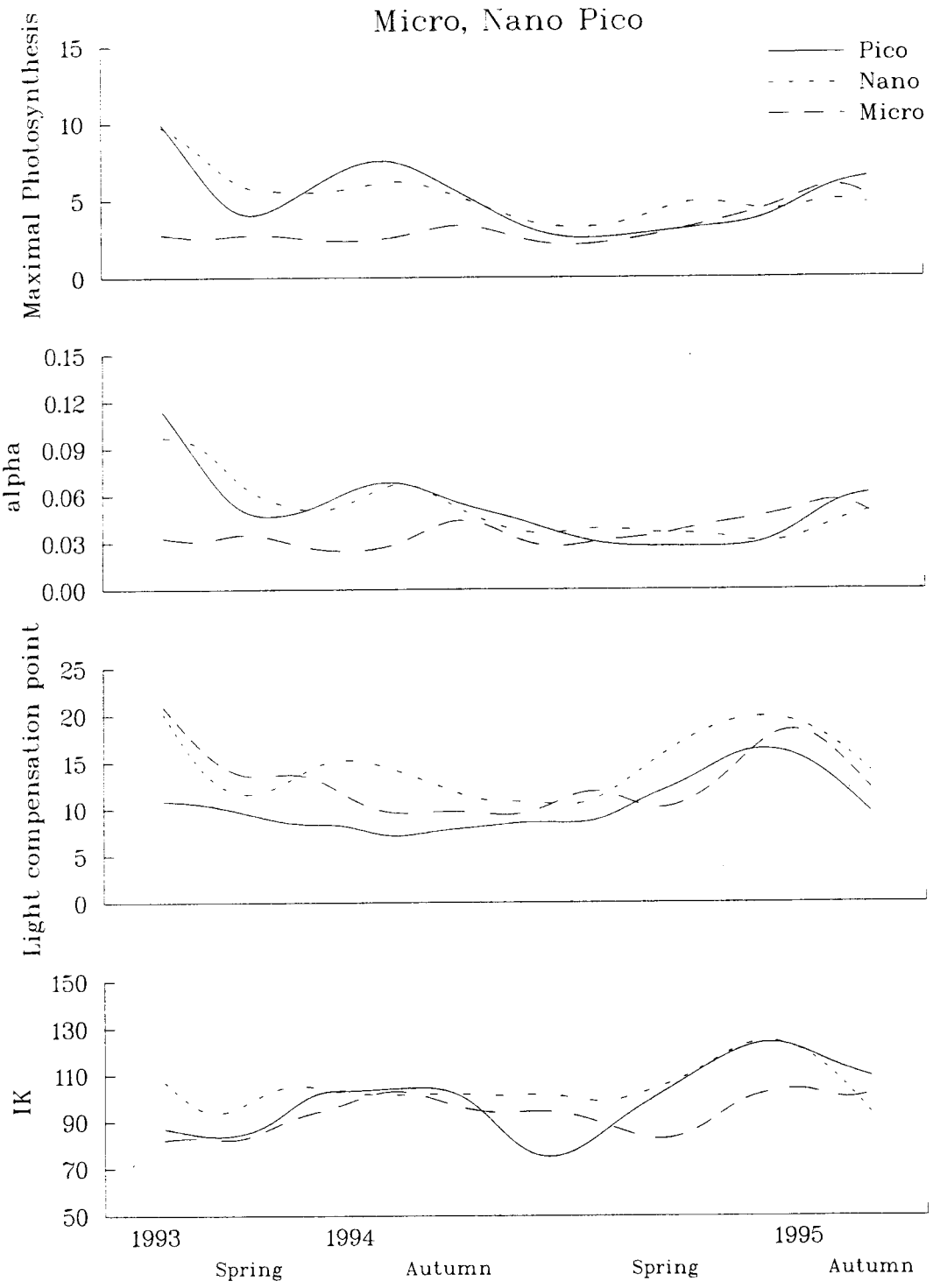


Figure 27: **Smoothed data from Figures 22-24, summarising seasonal changes in the photophysiological parameters on the different size fractions. Units as for figure 23.**

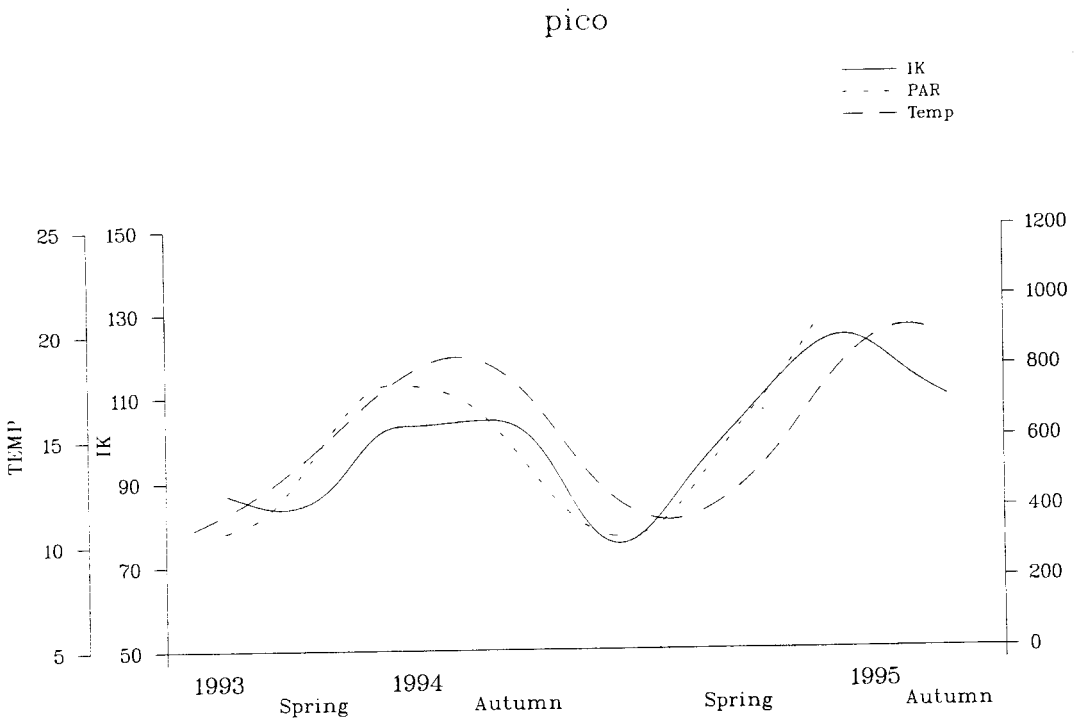


Figure 28: Superimposition of seasonal data for Ik of the pico fraction and temperatures and average photon flux over the previous 24 h. No such close correlation was observed with any other of the parameters/fractions. Units as for Figure 23.

3.7.3. Light as a factor limiting primary production

The parameter Ik is the light intensity at which carbon fixation first becomes light saturated. Whether or not cells are fixing carbon at light saturated rates is dependent on Ik, incoming photon flux, water column light attenuation and depth. By determining the Ik, the incoming photon flux and water column light attenuation, it is possible to estimate the proportion of daylight time when light is sufficient to saturate carbon fixation. Results of these calculations are shown in Figure 29. On average in Port Phillip Bay, there is sufficient light available for light saturated carbon fixation throughout the water column, for about 20% of the time. There is, however, no relationship between season and the proportion of time/water column that cells are fixing carbon at light saturated rates, reflecting the complicated nature of the responses of phytoplankton to environmental factors such as light. There was no significant difference between the behaviour of different phytoplankton size fractions in this respect and thus no support for the contention that the picoplankton might be less light limited than the larger species due to their superior efficiency of light harvesting (Morel & Bricaud 1981).

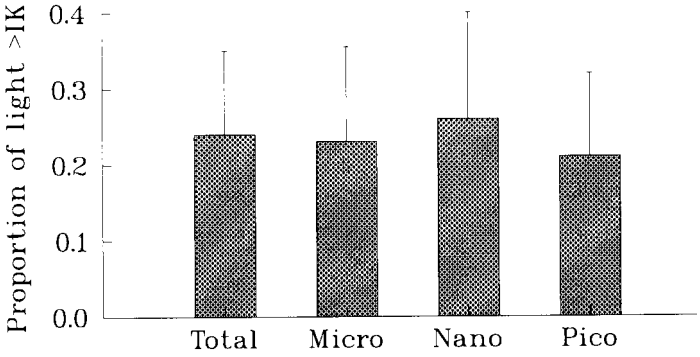


Figure 29: The proportion of time, based on daylight hours and depth, for which phytoplankton in Port Phillip Bay are exposed to theoretically saturating photon flux. All fractions spend 20-25% of the time at saturating photon flux.

3.7.4. *In situ* water column productivity

The preceding analyses have concentrated on the physiological parameters of photosynthesis of the phytoplankton in Port Phillip Bay. In this section, those parameters have been used, together with data on water depth at each station, incident photon flux and light attenuation through the water column, to produce depth integrated values for *in situ* water column primary productivity.

Water column daily carbon fixation rates for the total population are presented in Figure 30. Areal carbon fixation rates at any given station (Fig 30a) have higher values mostly during summer and early autumn and lower values during winter and late autumn. Estimates from one month to the next are also highly variable. Summer/autumn highs are in excess of 400 mg C m^{-2} day^{-1} whereas low values, which are mostly recorded in winter, are less than 200 mg C m^{-2} day^{-1}. This pattern is clearly shown by the Bay-wide mean daily carbon fixation rates (Figure 30b). Differences between stations are not extreme except that stations 3, 4 and 5 appear to support values in excess of 400 mg C m^{-2} day^{-1} over a number of consecutive sampling occasions (^{a}months) whereas this does not occur at other stations.

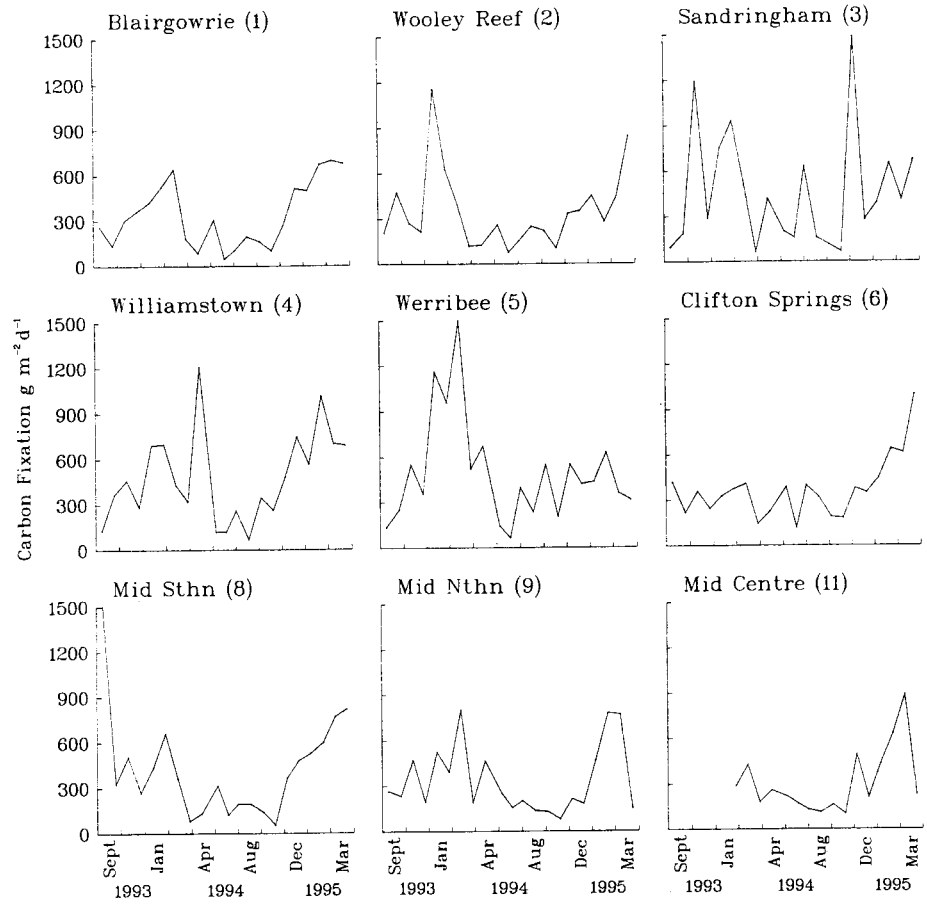
Daily fixation rates for the micro fraction are shown in Figure 31. Fixation rates considered high are those in excess of 100 mg C m^{-2} day^{-1} whereas low values are less than 100 mg C m^{-2} day^{-1}. The seasonal patterns tend to differ from station to station (Figure 31a) with a number of these patterns worth noting. Stations 2, 4 and 8 have summer/autumn peaks and winter lows. Station 6 shows autumn/winter peaks whereas station 5 shows spring/summer peaks and a winter low. The pattern at station 9 appears to be a lagged version of station 5 with a winter peak followed by a minimum in late winter/early spring. The percentage contribution of the micro fraction generally follows the carbon fixation rate with the overall correlation between the percentage contribution to total carbon fixation and the micro fraction carbon fixation rate yielding an r^2 value of 0.58. At individual sites the percent contribution varies from less than 10 % to around 80 %. Mean values for each sampling occasion show values in excess of 100 mg C m^{-2} day^{-1}, mostly in spring 1993 and summer/autumn for both years, and values less than 100 mg C m^{-2} day^{-1} in winter and/or spring 1994 (Figure 31b). The mean percentage contribution for all sites by the micro fraction is between 0% and 60% with most values less than 40% of total fixation.

The seasonal pattern of carbon fixation for the nano fraction appears similar to that for the total population (Figure 32). High values of greater than 100 mg C m^{-2} day^{-1} are most commonly recorded in summer and/or autumn and low values of less than 100 mg C m^{-2} day^{-1} are mostly recorded in winter and/or spring. Although the differences between stations (sites) were not large, a number of features are of note (Figure 32a). Carbon fixation rates at station 6 lack maxima and minima that are associated with season. At sites 9 and 11, winter minima extend over a number of months, whereas at stations 3,4 and 5, winter minima last for shorter periods and appear to be more erratic. In addition, high values occur over a number of months at sites 3, 4 and 5 whereas this did not seem to occur at other stations. The seasonal pattern of mean daily carbon fixation rates (Figure 32b) is one where values in excess of 100 mg m^{-2} day^{-1} occur in summer and/or autumn with lower values of less than 100 mg C m^{-2} day^{-1} occurring during winter. The relationship between percentage contribution to total production by the nano fraction and the nano fraction carbon fixation rate is not as strong as that shown by the micro fraction, with the r^2 being 0.29. The percentage contribution by the nano fraction to total production is thus much less variable than the equivalent data for the micro fraction. Mean values are between 20 and 60 % with most values around 40%.

The general pattern in daily carbon fixation rates for the pico fraction is similar for all sites (Figure 33a). High values in excess of 100 mg C m^{-2} day^{-1} occur in summer/autumn whereas low values less than 100 mg C m^{-2} day^{-1} occur in winter and/or early spring. A number of the mid-Bay group of stations have similarities in the patterns of carbon fixation. Stations 1, 8 9 and 11 appear to have winter minima that are consistent and occur over a longer period than at the other stations. The seasonal pattern for mean daily carbon fixation (Fig 33b) is one in which values greater than 100 mg C m^{-2} day^{-1} occur in summer/autumn and low values of less than 100 mg C m^{-2} day^{-1} occur during winter and early spring. The relationship between pico fraction daily carbon fixation and this group's percentage contribution to total carbon fixation shows a fairly weak relationship with an r^2 of 0.18. The contribution of pico fraction carbon fixation to the total is generally between 20 and 60% at any station. The mean percentage contribution to total fixation by the pico fraction is the least variable of any group with values mostly around 40%.

These results are consistent with other studies in the literature. There have been numerous studies of phytoplankton production and the contribution of different size fractions to total production. One factor that complicates comparisons is that different studies have used filters of different pore sizes to define the fractions. Despite this there are a number of features that seem to occur commonly. In many cases small cells, either <2 mm (Jiao and Wang 1994, Furnas and Mitchell 1987) or < 10mm (Sellner 1983) or < 20 mm (Abreu 1994, Jiao and Wang 1994, Revelante and Gilmartin 1978) make the dominant contribution to estimates of water column primary production. Cells larger than 20 mm appear to make sporadic intense contributions to water column production (Larsson and Hagstrom 1982, Harris *et al.* 1987, Malone *et al.* 1991, Jiao and Wang 1994). This certainly seems to be the case in Port Phillip Bay.

(A) Total population areal carbon fixation



(B) Bay wide mean areal Carbon fixation

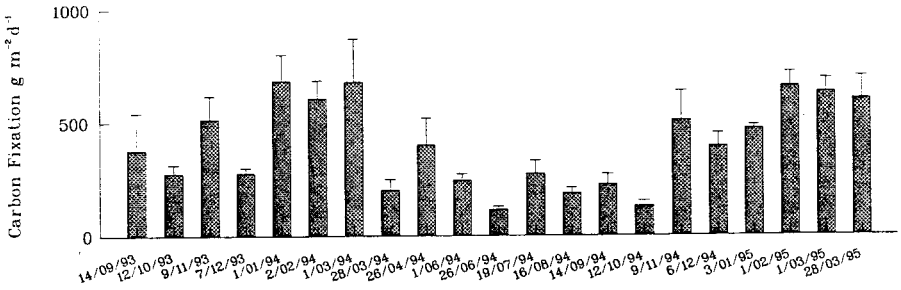


Figure 30: Areal based rates of carbon assimilation by the total phytoplankton population during the study period. [A] Data by station; [B] Bay-wide averaged data.

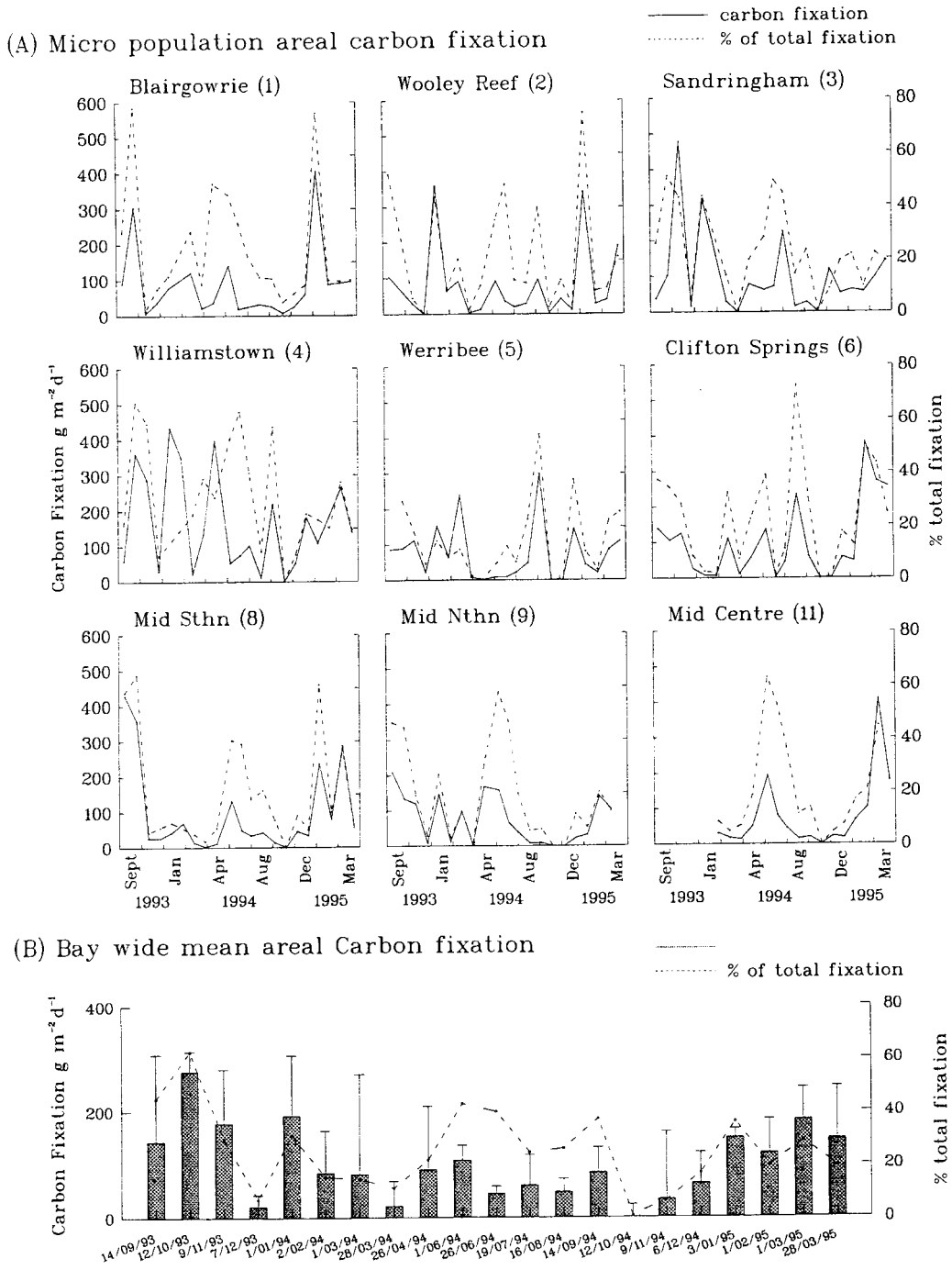


Figure 31: Areal based rates of carbon assimilation by the micro fraction during the study period. [A] Data by station; [B] Bay-wide averaged data.

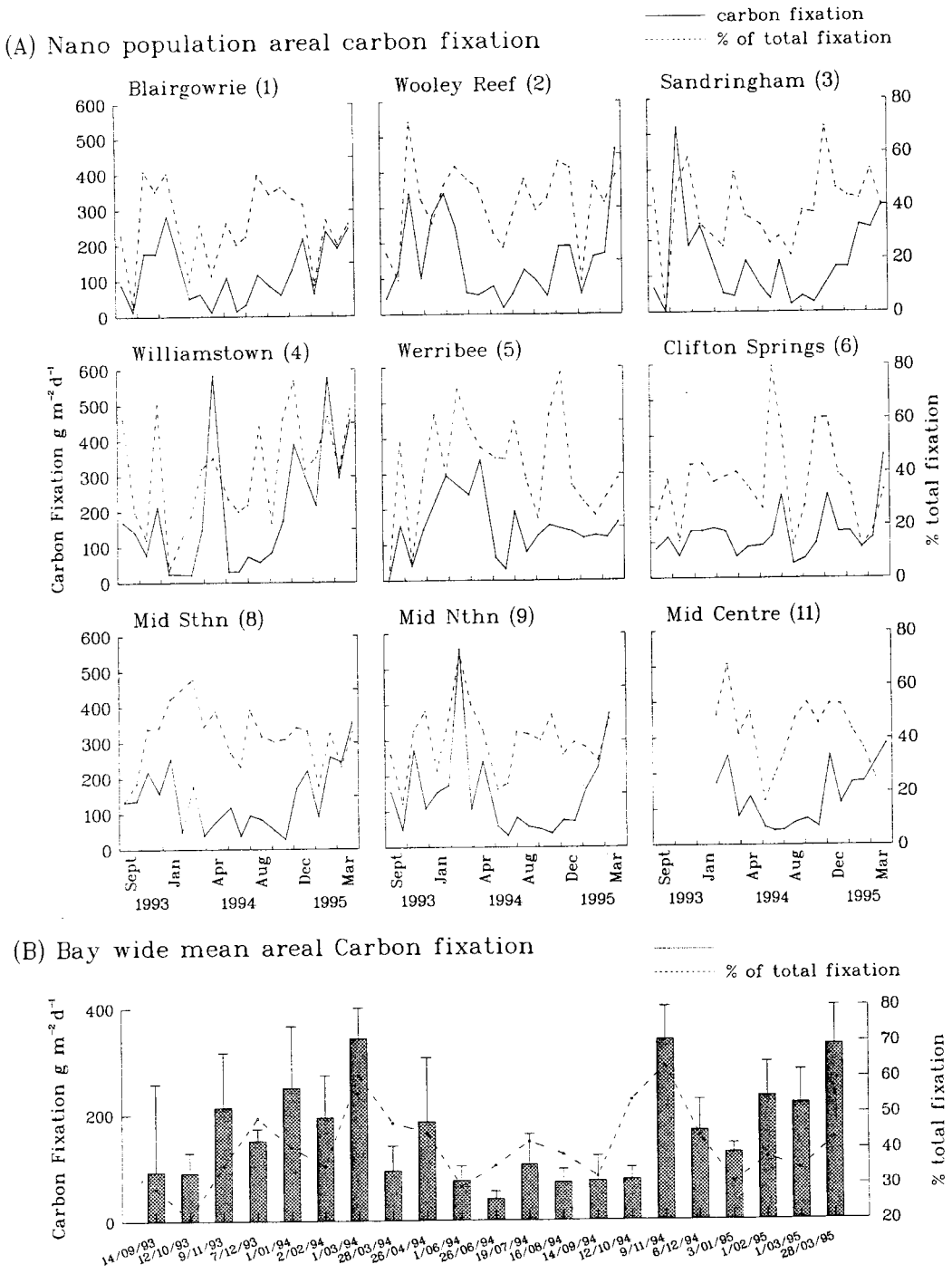


Figure 32: Areal based rates of carbon assimilation by the nano fraction during the study period. [A] Data by station; [B] Bay-wide averaged data.

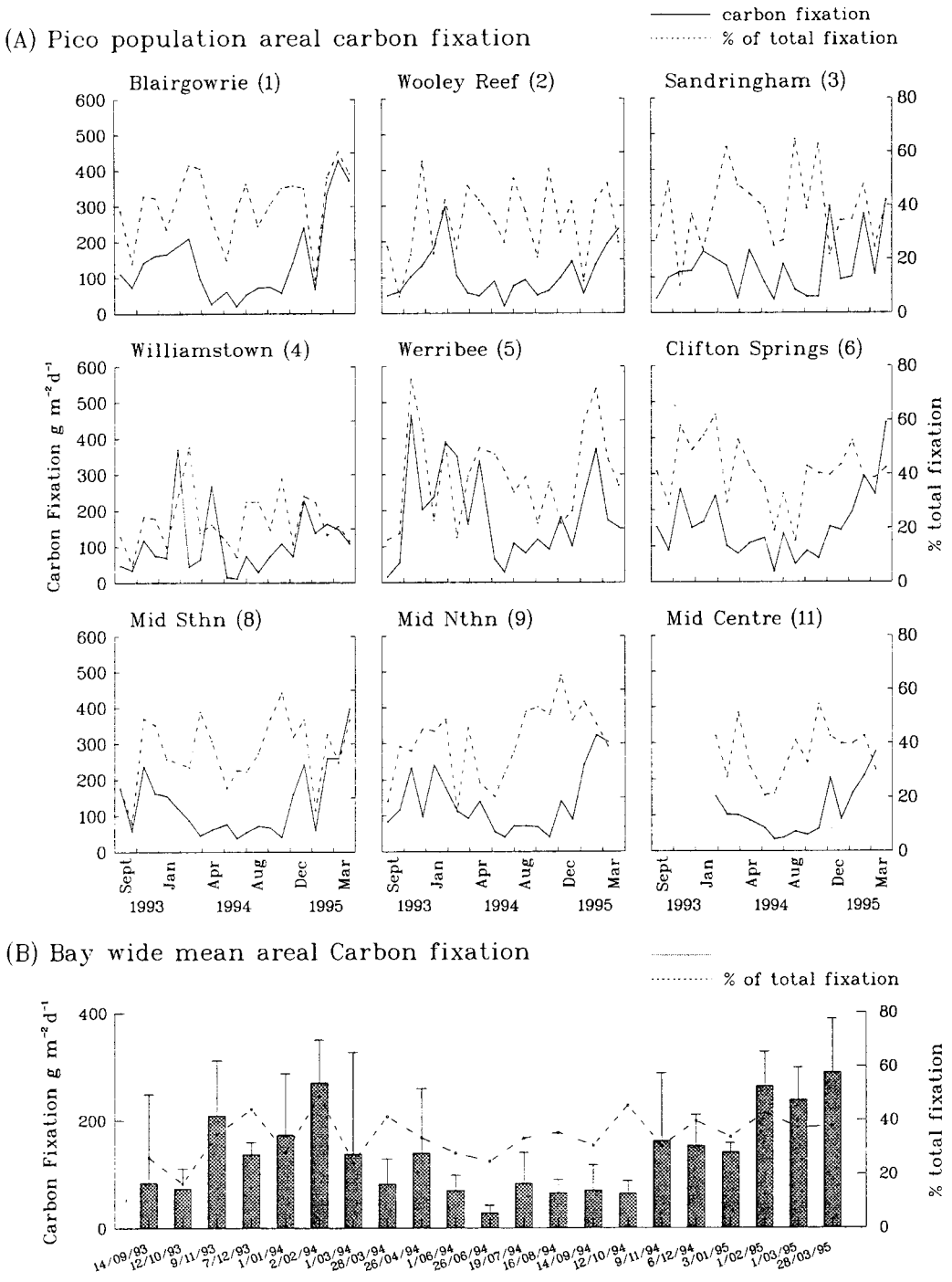


Figure 33: Areal based rates of carbon assimilation by the pico fraction during the study period. [A] Data by station; [B] Bay-wide averaged data.

3.8. An analysis of variations in biomass and productivity over different time and space scales

It is clear from the data presented in the foregoing sections that there can be considerable variation both in biomass and productivity between stations and sampling times. It is important therefore to establish that measured differences between sampling months or different stations are characteristic of the real spatial and temporal changes occurring in the Bay. To this end, we examined small scale variability on a number of cruises (see Table 2). Figure 34a -d shows examples for various components of the phytoplankton, of the variation in chlorophyll *a* and assimilation number in relation to space and time. It is clear that quite large variations may occur at a given station over a time scale of months (Figure 34 c) or even days (Fig 34 d). In some instances (Fig 34 a) small scale spatial variation was high. In Fig 34, data is presented only for some stations, fractions and cruises, but similar results were obtained on all "small scale sampling" trips.

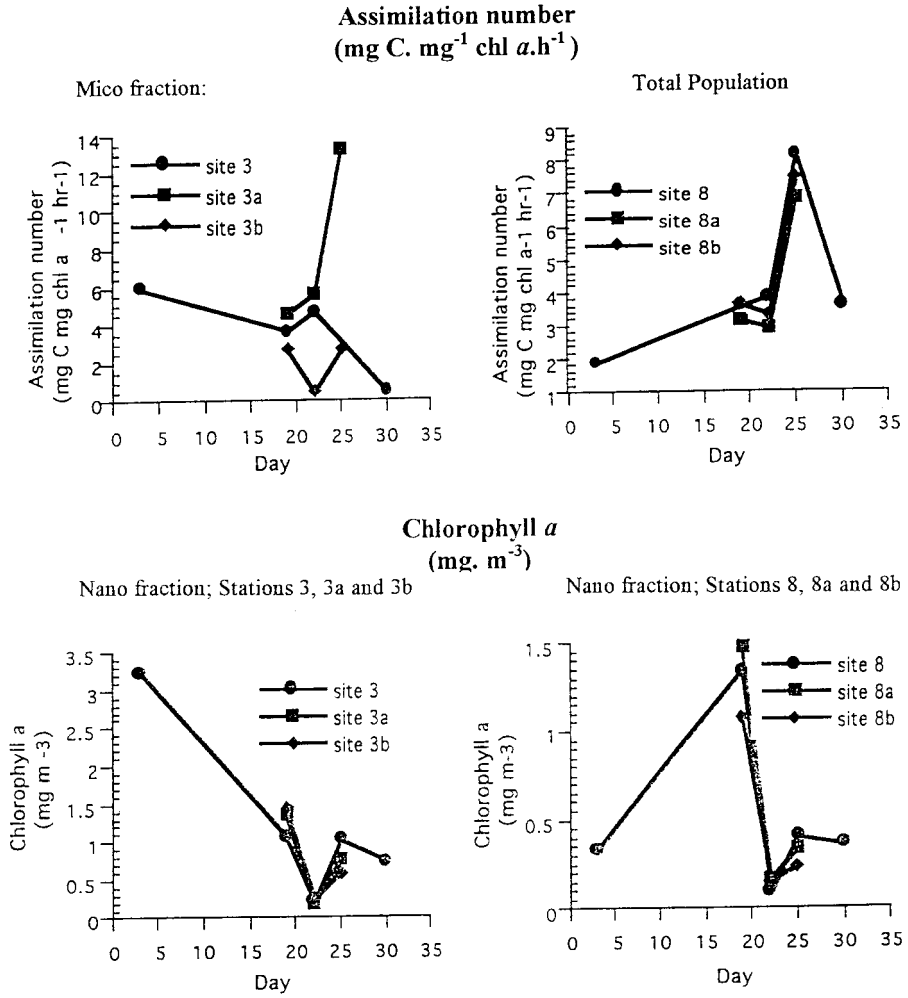


Figure 34: Variability in assimilation number (upper) and chlorophyll *a* (lower) during short time intervals (several days) and at different sub-stations (see Figure2). Data are from November/ December 1993.

In order to better quantify the extent and significance of this small scale variability, we have calculated the coefficient of variance for various parameters and size fractions. The levels of variation in light saturated carbon fixation (assimilation number) for the total population and each of the fractions was determined for a number of different time and space scales. The absolute size of the variance tended to increase with increases in the mean. Therefore, in order to assess variation relative to the mean, variability was quantified using the coefficient of variation (CV). Results of these analyses are shown in Figures 35 and 36.

For biomass, the coefficient of variation was much greater, for all fractions, at a scale of 10s of kilometres (Figure 35c) than at a scale of kilometres (Figure 35a). This difference was also present, though less apparent for assimilation number with the exception of the micro fraction which was just as variable at the smaller spatial scale (Figure 36 a) as at longer distances (Fig 36c). This suggests that for most fractions, sampling at the scale of the stations used in the present study can be used to give a satisfactory view of spatial distributions in chlorophyll, and also possibly assimilation number, around the bay. The high coefficient of variation for the micro fraction compared to the total population and the small size fractions confirms the inherent variability of the larger components of phytoplankton populations generally (Smetacek 1985; Søndergaard et al 1991; McQuoid & Hobson 1995).

Similar arguments can be made for the temporal scales. The higher coefficient of variation at a scale of months, for both chlorophyll (Figure 35d) and assimilation number (Figure 36d) confirms that the use of a monthly sampling regime will catch the main features of seasonal variation within the Bay. The variability of the micro fraction is further confirmed from their significantly higher coefficients of variation at different temporal scales compared to the other fractions.

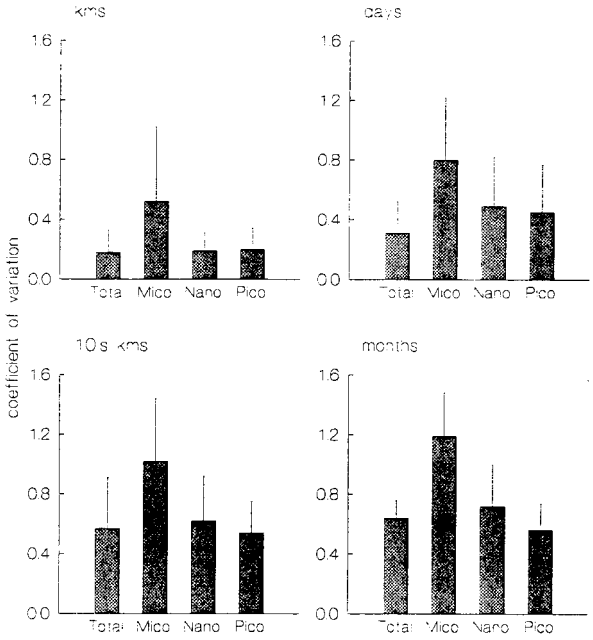


Figure 35: Coefficient of variation in chlorophyll *a* concentrations measured over time scales of kilometres (a) vs 10's of kilometres (c), or days (b) vs months (d).

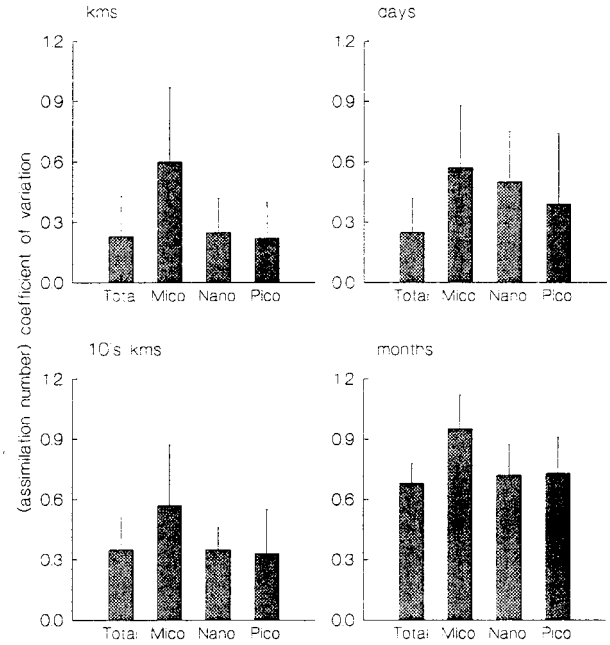


Figure 36: Coefficient of variation in assimilation number measured over time scales of kilometres (a) vs 10's of kilometres (c), or days (b) vs months (d).

3.9. Estimates of Bay-wide productivity

Seasonal summaries of mean biomass, areal water column daily primary productivity and biomass corrected primary productivity (assimilation number) are shown for "central" and "high" chlorophyll stations in Figures 37, 38, 39 and 40 for the total, micro, nano and pico fractions respectively. These summaries are based on the data collected for the 12 month period from spring 1994 to summer 1994/5.

For the total population (Figure 37), average water column biomass (chl *a* m^{-2}) was fairly constant throughout the year with only the spring values at central (low) stations showing biomass values significantly less than those of the yearly average (19.4 mg m^{-2}). Daily carbon fixation was greatest in summer and autumn for both central and coastal (high) stations, although at coastal stations, high fixation rates were also found in spring. Biomass corrected average daily *in situ* rates of carbon fixation ("Assimilation" in Figure 37 c) were lowest in winter and high, but similar, in all other seasons.

There were differences, between the coastal and central stations, in seasonal patterns of mean daily carbon fixation (Figure 41). During winter, both groups of stations showed low daily fixation of approximately 200 g C m^{-2} day^{-1}. In the coastal stations, fixation rates increased in spring to around 470 g C m^{-2} day^{-1} but rates remained low at this time in the central Bay. In summer, however, fixation rates at the central stations rose to values similar to those present in the coastal group (430 - 440 g C m^{-2} day^{-1}). Autumn saw fixation rates in the central stations drop to 337 g C m^{-2} day^{-1} but, at the coastal stations, values increased to 635 g C m^{-2} day^{-1}. Thus coastal stations had an extended period of high productivity over spring, summer and autumn whilst at the deeper, central, stations, daily fixation rates stayed low until a peak in summer, declining again in autumn.

The micro population showed considerable variation in mean seasonal biomass (Figure 38), with minimal levels at central stations in spring and highest levels during winter. In contrast, coastal stations had lowest biomass during summer and autumn, with maximal values in winter and spring. Mean daily carbon fixation rates were lowest in spring at the central stations (Figure 38) and highest during summer. In contrast, the coastal stations showed much more constant daily rates of fixation across the seasons, although values in summer and autumn tended to be higher than during spring and winter. Biomass corrected daily assimilation rates were highest for both groups of stations during summer, and lowest in winter (Figure 38).

The nano and pico fractions showed similar trends. Biomass was fairly constant throughout the year (Figures39, 40), the nano fraction showing slightly higher biomass during autumn than at other seasons. Daily mean carbon fixation on the other hand was far greater during summer and autumn and, for the nano fraction at coastal stations, fixation rates were high also during spring (Figs 39, 40). Consequently biomass adjusted assimilation rates were equally high in spring, summer and autumn for both fractions, with winter values being significantly lower (Figures 39, 40).

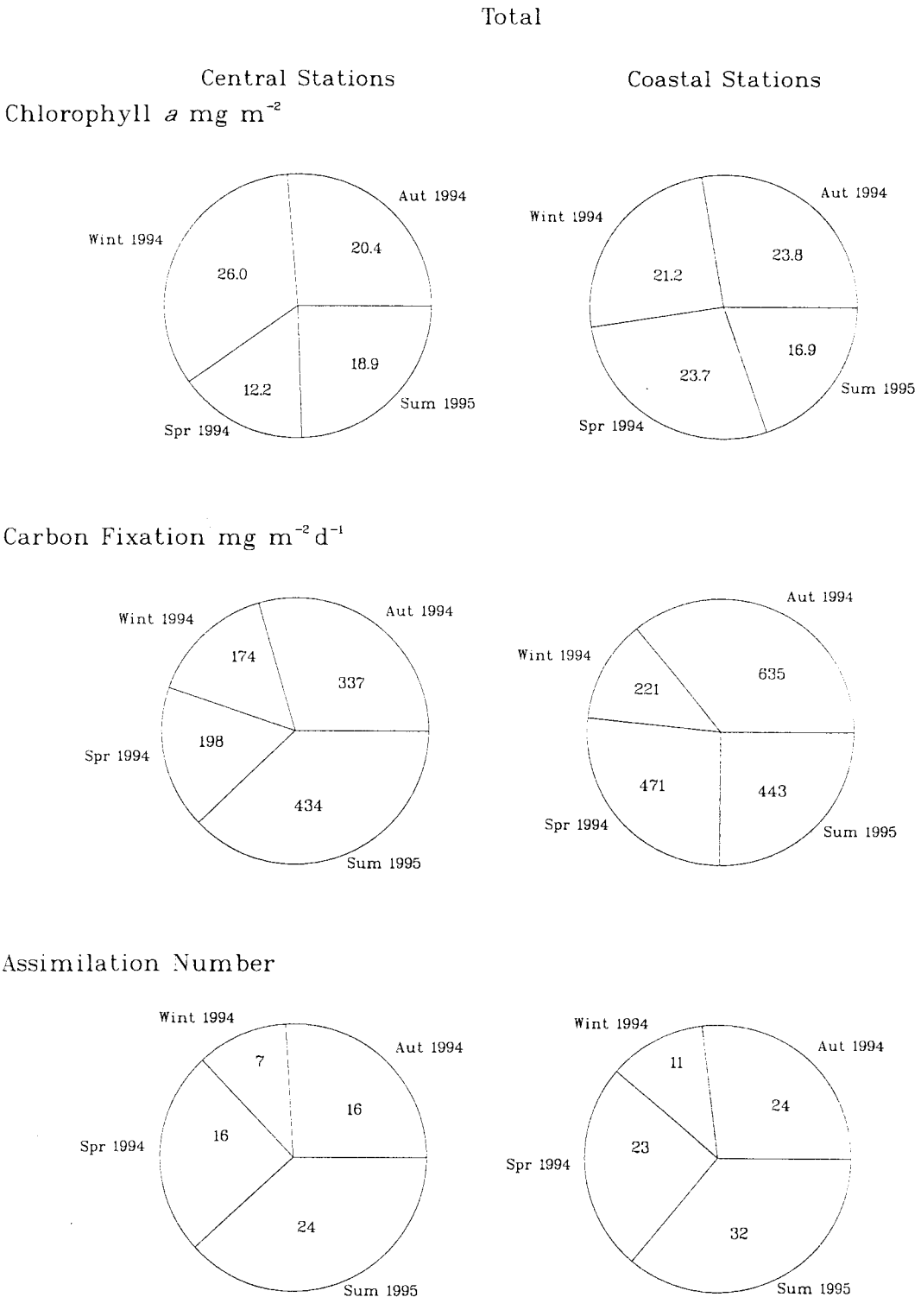


Figure 37: Pie charts showing, for the total phytoplankton population, the proportion of the average areal based chlorophyll a (upper), daily fixation of carbon (middle) and assimilation number (lower) attributable the different seasons. Numbers refer to the average values for a particular season.

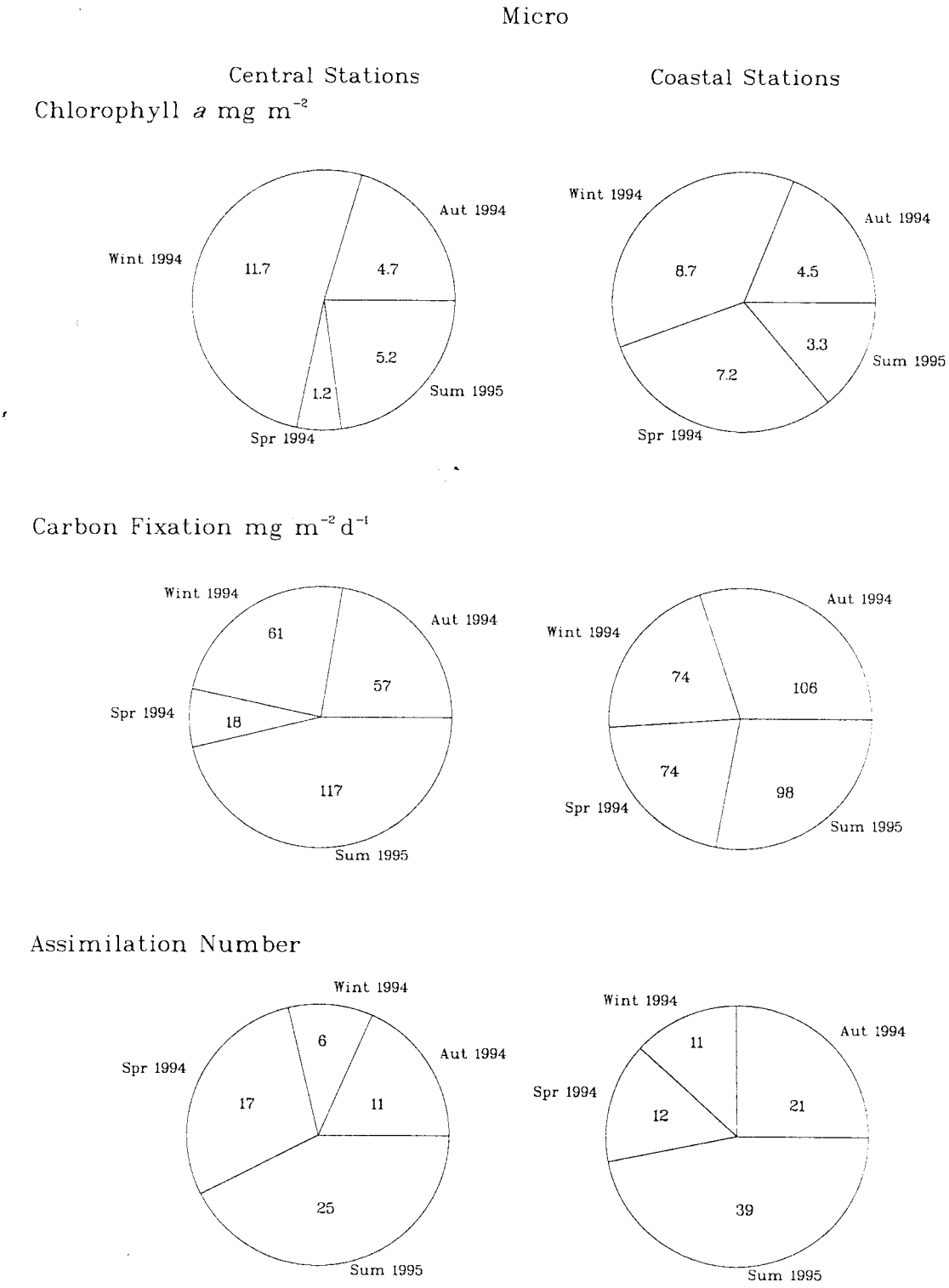


Figure 38: Pie charts showing, for the micro fraction, the proportion of the average areal based chlorophyll a (upper), daily fixation of carbon (middle) and assimilation number (lower) attributable the different seasons. Numbers refer to the average values for a particular season. Data are given for low chlorophyll (central) stations (left) and for high chlorophyll (coastal) stations (right).

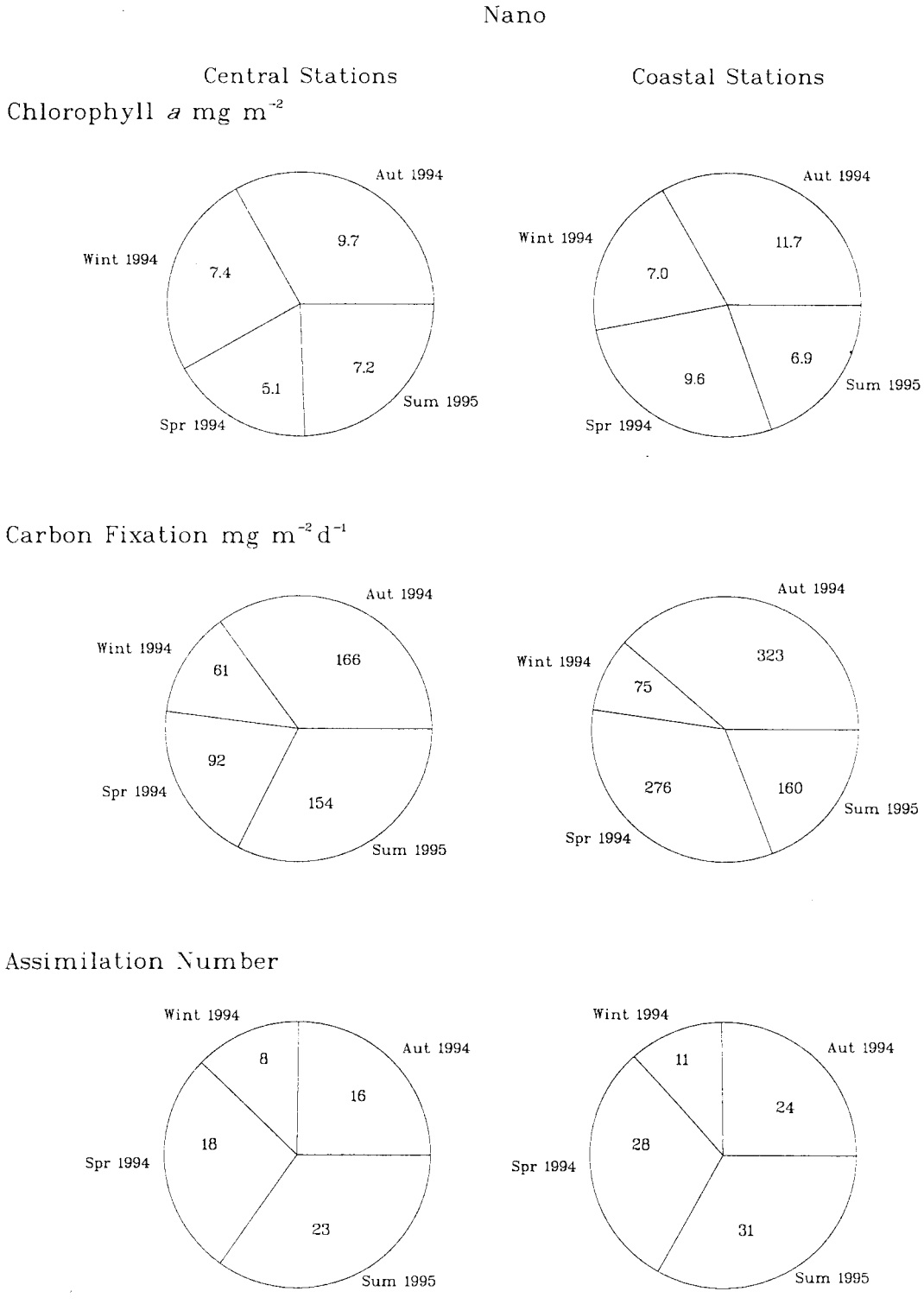


Figure 39: Pie charts showing, for the nano fraction, the proportion of the average areal based chlorophyll a (upper), daily fixation of carbon (middle) and assimilation number (lower) attributable the different seasons. Numbers refer to the average values for a particular season. Data are given for low chlorophyll (central) stations (left) and for high chlorophyll (coastal) stations (right).

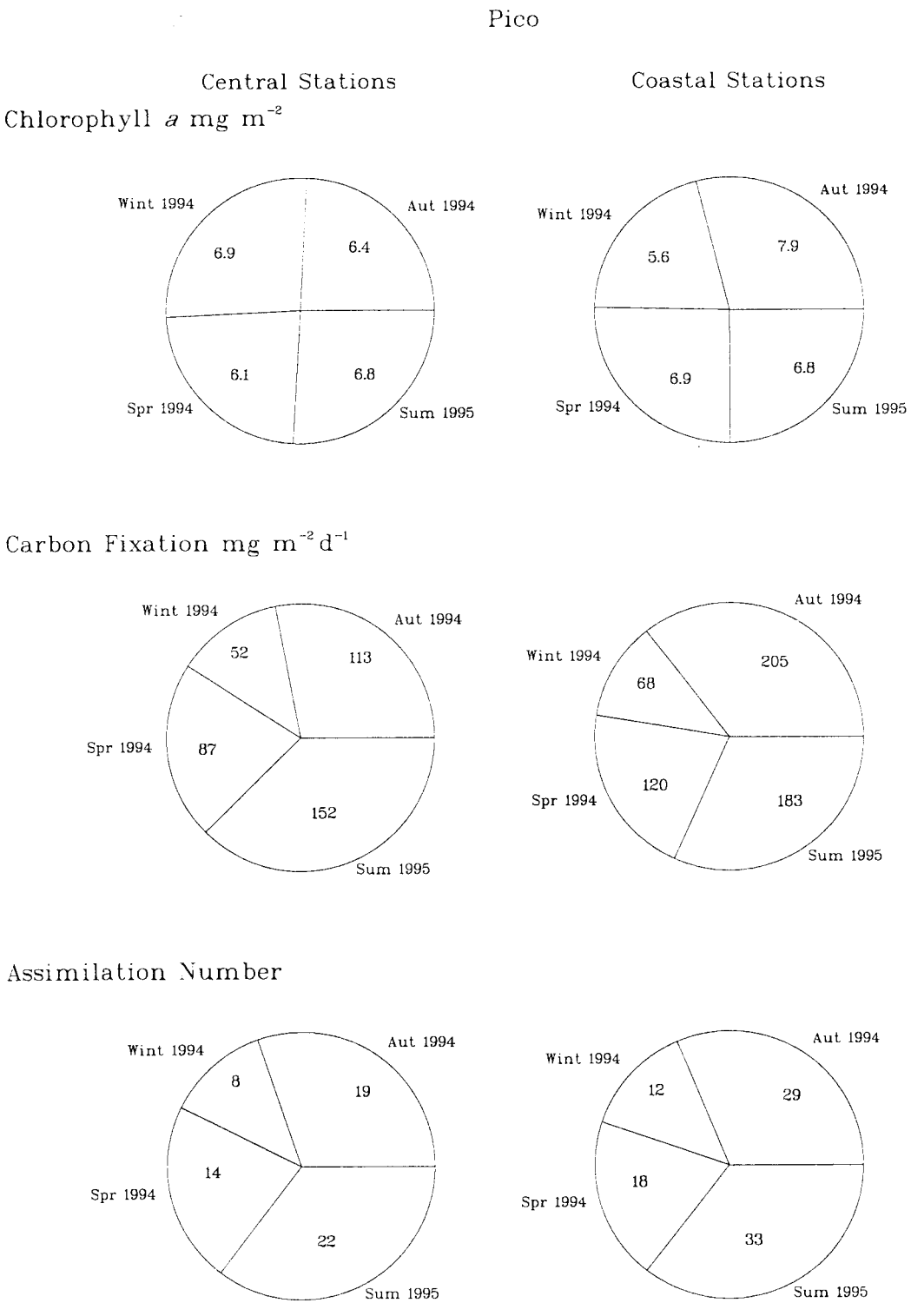


Figure 40: Pie charts showing, for the pico fraction, the proportion of the average areal based chlorophyll a (upper), daily fixation of carbon (middle) and assimilation number (lower) attributable the different seasons. Numbers refer to the average values for a particular season. Data are given for low chlorophyll (central) stations (left) and for high chlorophyll (coastal) stations (right).

The average daily carbon fixation data confirm the seasonal variability of the micro fraction which contributed between 14% and 34% of the water column primary productivity. On the other hand, the pico fraction appears a much more constant contributor to the overall primary productivity of Port Phillip Bay with daily average fixation rates varying seasonally from 30.3% to 35.8% of the total production. The nano fraction contributed from 34% to 55% of the daily primary productivity.

These observations confirm that Port Phillip Bay is similar to a number of other marine ecosystems in which a stable biomass and primary productivity contributed by the smaller size class species (picoplankton and, to a lesser extent, nanoplankton) is overlaid by a much more variable micro population.

The foregoing information can be used to draw up an annual budget for primary productivity in Port Phillip Bay. This data is summarised in Table 10.

Thus, approximately 260,000 tonnes of C is sequestered annually by the phytoplankton in Port Phillip Bay. Using the Redfield ratio, this translates into the assimilation of approximately 43,000 tonnes of N per year. Of the carbon fixed, 170000 tonnes, or 65.61%, is fixed by the nano- and micro-phytoplankton. These organisms will tend to sediment out of the water column more readily than the smaller pico fraction. If we again apply Redfield stoichiometry to these figures, this means that approximately 28,000 tonnes of N would be assimilated by the larger phytoplankton and, potentially, rapidly return to the sediment. It is perhaps interesting to note here that this figure is considerably larger than estimates for the annual loading of inorganic nitrogen into the Bay (6,000 - 8,000 tonnes). If we assume that the nano fraction will not sediment as readily as the micro fraction, then the figure for N settling out reduces to 8,970 tonnes per year.

Table 10: Annual productivity of the phytoplankton in Port Phillip Bay.

Season	Fraction	Mean daily C assimilation rate ($g.m^{-2}.day^{-1}$)	Annual productivity (10^3 tonnes C . $season^{-1}$)
Winter	Micro	68	12.01
	Nano	68	12.10
	Pico	60	10.68
	Total	198	***35.23***
Spring	Micro	46	8.19
	Nano	184	32.74
	Pico	104	18.42
	Total	335	***59.52***
Summer	Micro	107.5	19.13
	Nano	157	27.94
	Pico	167.5	29.80
	Total	438.5	***78.03***
Autumn	Micro	81.5	14.50
	Nano	244.5	43.51
	Pico	159	28.29
	Total	486	***86.48***
	Total Annual Production (tonnes C)		**259.26**

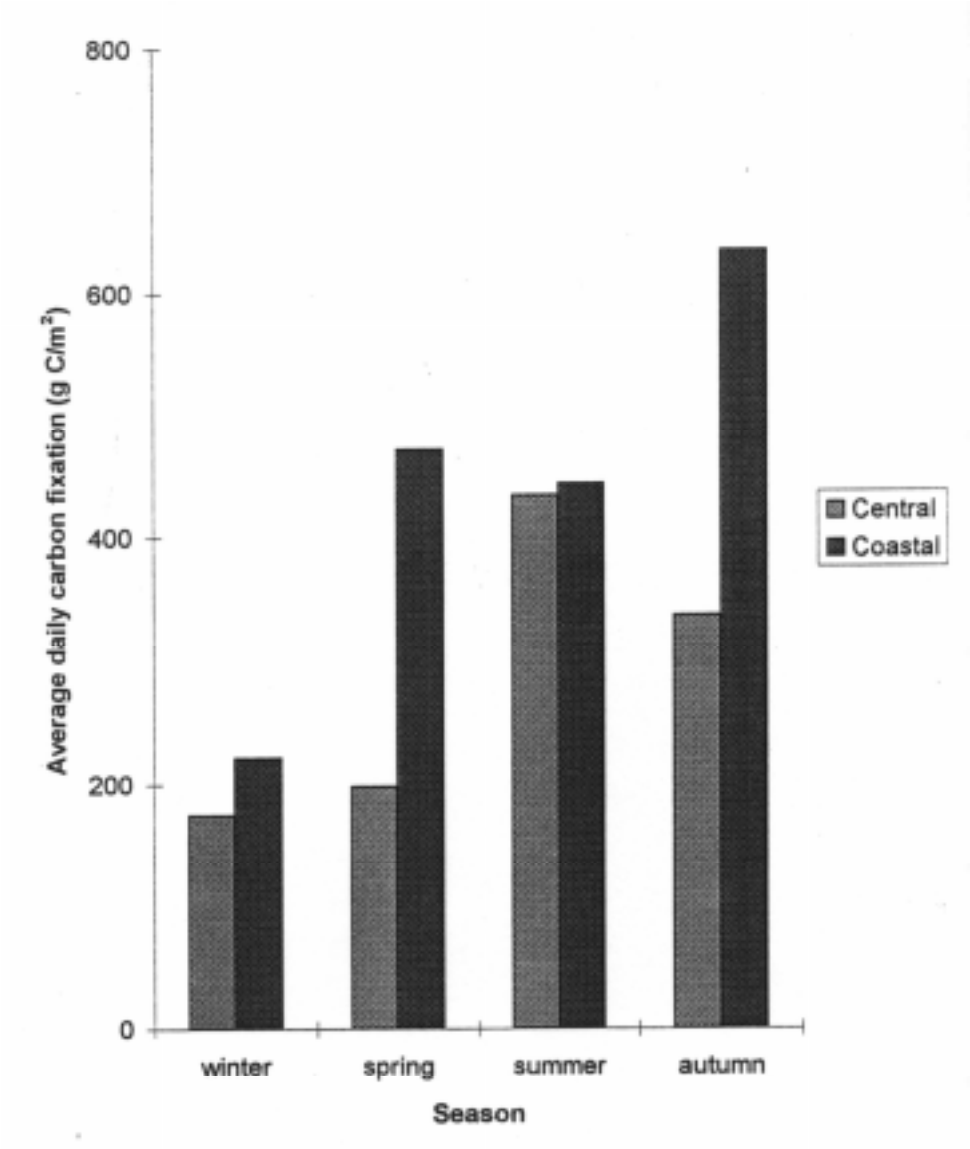


Figure 41: Bay wide estimates for the average daily rates of carbon fixation in central (grey bars) or coastal (black bars) stations in Port Phillip Bay. Data are presented by season.

4. SUMMARY AND RECOMMENDATIONS FOR FUTURE MONITORING.

Port Phillip Bay supports a phytoplankton population which is not particularly large compared to other shallow marine ecosystems. In common with many other systems however, it is apparent that the biomass and productivity of the phytoplankton are characterised by a relatively stable contribution (30-40%) from the picoplankton and, to a less marked degree, the nanoplankton. On top of this is superimposed a highly variable population of larger celled organisms, the microphytoplankton.

From the studies carried as part of this project and reported here, some points regarding future monitoring of the phytoplankton of the Bay suggest themselves.

1. It is clear that given the importance of the smaller components of the phytoplankton, any future sampling program must take into account the contributions made by the pico and nano fractions. Samples for estimation of total chlorophyll should be collected on membrane filters (0.2m) or, if this is not possible, on glass fibre filters with smaller pore diameter (e.g.GF/F)
2. The relative contribution of the three cell sizes to total chlorophyll should continue to be monitored, separate size fractionated samples be dark adapted and F/Chl measured. If possible, institute a study on the effect of light on the F/Chl in each class.
3. Either total or size fractionated samples should be dark adapted and F/Chl, together with extracted chlorophyll, be measured. If possible, a number of separate samples should be taken from a range of depths at selected stations to investigate the degree of probability that nutrient status is influenced by flux from the benthos.
4. Coastal vs central stations: It is clear from the data reported here, that coastal and central stations form distinct groups. Any future sampling program should take this into account
5. Monthly sampling will cover most major events
6. Given that the pico fraction can comprise a constant 40% of carbon assimilation, future measurements of carbon assimilation must include the smaller size fractions which are sometimes excluded in studies using GF/C filters (see 1).

5. ACKNOWLEDGEMENTS.

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6. REFERENCES

Abreu, P.C., Odebrecht, C. and Gonzalez, A., 1994. Particulate and dissolved phytoplankton production in the Patos Lagoon estuary, southern Brazil: comparison of methods and influencing factors. *J. Plankton Res.*, 16(7): 737- 753.

Alpine A.E. and Cloern, J.E. 1985. Differences in *in-vivo* fluorescence yield between three phytoplankton size classes. *Journal of Plankton Research* : 381-390

Arnott, G.H., 1990. Surveillance of toxic marine algae and biotoxins in shellfish in Port Phillip Bay. Marine Science Laboratories, Queenscliff, Internal report No. 187, Department of Conservation & Environment, Fisheries Management Division.

Axelrad, D.M., Hickman, N.J. and Gibbs, C.F.,1979. Microalgal productivity as affected by treated sewage discharge to port Phillip Bay. Environmental Studies Series, Publication No. 241, Ministry for Conservation, Victoria.

Axelrad, D.M., Poore, G.C.B., Arnott, G.H., Bauld, J., Brown, V., Edwards, R.R.C. and Hickman, N.J., 1981. The effects of treated sewage on the biota of Port Phillip Bay, Victoria, Australia. *In:* Neilson, B.J. and Cronin, L.E. (eds) *Estuaries and Nutrients* 1st ed., pages 279-306, The Humana Press, Clifton, New Jersey.

Cleveland J.S. and Perry, M.J. 1987. Quantum yield, relative specific absorbtion and fluorescence in nitrogen-limited *Chaetoceros gracilis. Marine Biology* (Berlin) 94: 489-497

Collos, Y., Descolas-Gros, C., Fontugne, M., Mortain-Bertrand, A., Chretiennot- Dinet, M.J. and Frikha, M.G., 1992. Carbon and nitrogen dynamics during growth and degradation of phytoplankton under natural surface irradiance. *Mar. Biol.*, 112: 491-496.

Falkowski, P.G. and Kolber, Z., 1995. Variations in chlorophyll fluorescence yields in phytoplankton in the world oceans. *Aust J. Plant Physiol.*, 22(2): 341-355.

Fitzwater, S.E., Knauer, G.A. and Martin, J.H., 1982. Metal contamination and its effect on primary production measurements. *Limnol. Oceanogr.*, 27:544- 551.

Furnas, M.J. and Mitchell, A.W., 1987. Phytoplankton dynamics in the central Great Barrier Reef. 2. Primary production. *Cont. Shelf Res.*, 7:1049-1062

Furnas, M.J. and Mitchell, A.W., 1988. Photosynthetic characteristics of Coral Sea picoplankton (<2mm size fraction). *Biol. Oceanogr.*, 5: 163-182.

Grant, B.R. and Gayler, K.R., 1982. Phytoplankton productivity study. Chapter 6- Supplementary Papers. *In:* Sandiford, R.N. and Holmes, N. (eds) *The Heated Effluent Study - Final Report* pages 255-326. Technical Report No. 15, Marine Science Laboratories, Victoria

Green, R.M., Kolber, Z.S., Swift, D.G., Tindale, N.W. and Falkowski, P.G., 1994. Physiological limitation of phytoplankton photosynthesis in the eastern equatorial Pacific determined from variability of quantum yield of photosynthesis. *Limnol. Oceanogr.*, 39(5): 1061-1074.

Guo C, Dunstan WM (1995) Depth-dependent changes in chlorophyll fluorescence number at a Sargasso Sea station. *Marine. Biology*. 122: 333-339

Hansen .B, Fotel, F.L., Jensen, N.J. and Madsen S.D. 1996. Bacteria associated with a marine planktonic copepod in culture. II. Degradation of fecal pellets produced on a diatom, a nanoflagellate or a dinoflagellate diet. *Journal of Plankton Research* 18: 275-288

Harris, G.P., 1986. Phytoplankton ecology: structure, function and fluctuation. Chapman and Hall, New York.

Harris, G.P., Ganf, G.G. and Thomas, D.P., 1987. Productivity, growth rates and cell size distributions of phytoplankton in the SW Tasman Sea: implications for carbon metabolism in the photic zone. *J.Plankton Res.*, 9(5): 1003-1030.

Jassby, A.D. and Platt, T.R., 1976. Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnol. Oceanogr.*, 21: 540-547.

Jaio, N. and Wang, R., 1994. Size structures of microzooplankton biomass and production in Jiaozhou Bay, China. *J. Plankton Res.*, 16(12): 1609-1625.

Jeffery, S.W. and Humphrey, G.F., 1975. New spectrophotometric equations for determining chlorophyll $a,b,c_1$and c_2 in higher plants , algae and natural phytoplankton. *Biochem. Physiol. Pflanzen.*, 167; 191-194.

Jitts, H.R., Morel, A. and Saijo, Y., 1976. The relation of oceanic primary production to available photosynthetic irradiance. *Aust. J. mar. Freshwat. Res.*, 27: 441-454.

Kirk, J.T.O., 1983. Light and photosynthesis in aquatic ecosystems. Cambridge University Press, Cambridge.

Kiefer, D.A. 1973. Chlorophyll *a* fluorescence in marine centric diatoms: Response of chloroplasts to light and nutrient stress. *Mar. Biol.* 23: 39-46

Kolber, Z., Wyman, K.D. and Falkowski, P.G. 1990. Natural variability in photosynthetic conversion efficiency: A field study in the Gulf of Maine. *Limnology and Oceanography* 35: 72-79

Larsson, U. and Hagstrom, A., 1982. Fractionated phytoplankton primary production, exudate release and bacterial production in a Baltic eutrophication gradient. *Mar. Biol.*, 67: 57- 70.

Lean, D.R. and Burnison, B.K., 1979. An evaluation of the errors in the ^{14}C method of primary production measurements. *Limnol. Oceanogr.*, 24: 917-928.

Longmore, A., Cowdell, R. and Gibbs, C., 1990. Monitoring of water quality in Port Phillip Bay, 1985-1986. (SRS 89/003) Environment Protection Authority, Victoria. 81 pages.

Malone, T.C. and Neale, P.J., 1981. Parameters of light dependent photosynthesis for phytoplankton size fractions in temperate estuarine and coastal environments. *Mar. Biol.*, 61: 289-297.

Malone, T.C., Ducklow, H.W., Peele, E.R. and Pike, S.E., 1991. Picoplankton carbon flux in Chesapeake Bay. *Mar. Ecol. Prog. Ser.*, 78: 11-22.

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

Morel A & Bricaud A, 1981. Theoretical results concerning light absorbtion in a discrete medium and application to specific absorption of phytoplankton. Deep Sea Res 28, 1375-93

Parsons, T.R., Maita, Y. and Lalli, C.M., 1984. *A Manual of Chemical and Biological Methods for Seawater Analysis*, Pergamon Press, New York.

Revelante, N. and Gilmartin, M., 1978. Characteristics of the microplankton and nanoplankton communities of an Australian coastal plain estuary. *Aust J. Mar. Freshw. Res.*, 29: 9- 18.

Sellner, K.G., 1983. Plankton productivity and biomass in a tributary of the upper Chesapeake Bay. I. Importance of size fractionated phytoplankton productivity, biomass and species composition in carbon export. *Estuar., cstl. Shelf Sci.*, 17: 197-206.

Sondergaard, M., Jensen, L.M. and Aertebjerg G (1991) Picoalgae in Danish coastal waters during summer stratification. *Marine Ecology Progress Series* 79: 139-149

Tiselius, P. and Kuylenstierna, M. 1996. Growth and decline of a diatom spring bloom: phytoplankton species composition, formation of marine snow and the role of heterotrophic dinoflagellates. *Journal of Plankton Research* 18 : 133-155

Wehr, J.D., Lee, J. and Campbell, L. 1994. Does microbial biomass affect pelagic ecosystem efficiency? An experimental study. *Microbial Ecology* 27: 1-17

Wood, M. and Beardall, J., 1992. Phytoplankton ecology of Port Phillip Bay, Victoria. Technical Report No. 8 CSIRO Port Phillip Bay Environmental Study. 39pp

Wood, M.D. and Oliver, R.L. 1995. Fluorescence transients in response to nutrient enrichment of Nitrogen- and Phosphorus-limited *Microcystis aeruginosa* cultures and natural phytoplankton populations: a measure of nutrient limitation. *Aust. J. Plant. Physiol*. 22: 331-340.