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Microzooplankton Grazing on Phytoplankton in Port Phillip Bay

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EXECUTIVE SUMMARY

The primary objective of this study was to estimate microzooplankton grazing impact on phytoplankton in Port Phillip Bay. The study was conducted over the period October 1994 to September 1995 and included monthly investigations using experimental water collected from mid-depth at four coastal and two midbay sites. The Dilution Method was employed to estimate grazing rates under laboratory conditions. Estimates of phytoplankton growth rate were also determined from the same experiments but interpretation of growth rates in this study is somewhat limited due to the use of an artificial light:dark cycle and the addition of dissolved inorganic nitrogen to incubated seawater samples. Estimates of phytoplankton growth without nutrient addition were determined using control bottles.

Seasonal patterns in total phytoplankton growth were apparent for all stations, with growth rates being highest during spring and summer and lowest during winter, for both nutrient enhanced and nonspiked treatments. Phytoplankton growth rates of the pico, nano and micro fractions were positively correlated with temperature when inorganic nitrogen was added. Without additional nutrients, only the pico fraction growth rates showed a positive relationship with temperature. Nonspiked growth rates of the nano and micro fractions were low during summer; relatively higher summer growth rates exhibited by the pico fraction indicate that they were better able to utilise available and regenerated pools of dissolved inorganic nitrogen at high temperatures and low ambient nitrogen concentrations.

Nutrient enhanced growth rate estimates were very similar to rates determined without additional nitrogen during the autumn and winter months, when ambient NH_4 levels were relatively high (0.4-1 μM). Because ambient nutrient levels appeared to be less limiting for microalgal growth during autumn and winter, estimates determined for this period may be good approximations of *in situ* growth rates.

Seasonal trends in grazing rate were apparent all sites, except Sandringham, with maximum grazing rates in summer (mean = 0.51 d^{-1}) and with low grazing impact in winter (mean = 0.13 d^{-1}). At most sites, grazing rates on the total phytoplankton population tended to mirror total chlorophyll *a* growth rates, with high grazing rates observed during peaks in chlorophyll *a* growth, and with reduced grazing rates when chlorophyll *a* growth rates were low.

The grazing coefficients tended to be higher for the pico and nano fractions than for the microphytoplankton suggesting that the microzooplankton grazers in Port Phillip Bay are most important in regulating phytoplankton cells <20 μm. Low but significant levels of grazing on the micro fraction were shown at some stations in summer, however, in 75% of experiments conducted during this study, estimates of grazing rate on the micro fraction were not significantly different from zero ($p<0.05$).

Though estimates of grazing impact determined in this study show much temporal and spatial variability, general seasonal trends suggest that, over all sites, the mean grazing impact on the initial phytoplankton standing stock was approximately 16% in spring, 38% in summer, 18% in autumn and 11% in winter.

Baywide estimates of grazing impact on potential production during summer averaged 48% when nitrogen was added and 82% when phytoplankton growth was not enhanced by nutrient addition. Mean estimates of grazing impact on algal production during autumn, winter and spring were in the range of 23-48% (nitrogen enhanced) and 47-64% (nonspiked) of daily production.

The grazing rates estimated in this study include the grazing impact of microzooplankters and some mesozooplankters and are thus more representative of the broader zooplankton community in Port Phillip Bay. Other mesozooplankton which may contribute greatly to the grazing impact in the Bay, and whose impacts need to be assessed, are the larvacean, *Oikopleura dioica*, and the polyphagous dinoflagellate, *Noctiluca scintillans*, a relatively new resident of Port Phillip Bay waters.

Overall, it appears that the microzooplankton are currently very important in controlling the phytoplankton population in Port Phillip Bay. Although an increase in microalgal growth rates, resulting from potentially larger inputs of nutrients to the Bay, could decrease the grazing impact of current concentrations of micrograzers, it is also possible that greater phytoplankton production may result in increases in the size of the zooplankton population in Port Phillip Bay.

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1. INTRODUCTION

1.1. Importance of Microzooplankton

Grazing by microzooplankton on phytoplankton is now widely recognised as significant in most aquatic ecosystems. The microzooplankton comprise abundant and diverse taxa which grow rapidly and which feed efficiently on cells primarily <20 μm (Gifford 1985, Verity 1985). They include consumers of both phytoplankton and bacterial production and, because of their abundance, contribute significantly to trophic flux and nutrient cycling (Sherr and Sherr 1984, Porter *et al.* 1985, Fenchel 1988). These small grazers can be effective in controlling the size of phytoplankton populations (Gifford 1988) and the size structure of the phytoplankton community (Verity 1986) and may also control, by selective predation, phytoplankton species composition (Burkill *et al.* 1987, Paranjape 1990). Pierce and Turner (1992) suggest that it is likely that the relative importance of the microzooplankton vs net zooplankton shifts seasonally as well as spatially with shifts in phytoplankton size structure.

The planktonic food web interactions and the role of microzooplankton in Port Phillip Bay has not been well understood. Earlier zooplankton studies, which are reviewed in Crawford *et al.* (1992) and Holloway and Jenkins (1993), provide information on the composition and distribution of primarily net zooplankton. References to the microzooplankton are few, and do not include abundances of heterotrophs <50 μm. Overall, the microzooplankton community has received little attention and its role in regulating the phytoplankton population in Port Phillip Bay has not been previously examined.

1.2. Overall Aims of the Study

This study (Task E.10.5) is the first attempt to quantify the impact of community microzooplankton grazing on phytoplankton in Port Phillip Bay. The main objectives of the study were:

1. to estimate the microzooplankton grazing impact on phytoplankton on a monthly basis at selected coastal and midbay sites in Port Phillip Bay,
2. to estimate grazing rates on the pico-, nano- and microphytoplankton fractions, and
3. to estimate phytoplankton growth rates, with and without additional inorganic nitrogen, for the total population and for the pico-, nano- and microphytoplankton fractions.

Estimates of zooplankton grazing impact determined from this study contribute to the understanding of the Bay ecology and to the ongoing development of a model of the physical, chemical and biological processes in Port Phillip Bay.

1.3. Study Sites

Microzooplankton grazing experiments were conducted at approximately four weekly intervals with seawater collected from mid-depth at six sites (four coastal and two midbay) in Port Phillip Bay (Figure 1, Table 1). A total of 41 laboratory grazing experiments were conducted from October 1994 until September 1995. Grazing rates for Wooley's Buoy (Stn 2) was investigated on a monthly basis; grazing rates for most other sites were examined bi-monthly.

Table 1. Station locations, total depth at each site and approximate depth from which seawater samples were collected.

Station	Station Number	Location	Site depth (m)	Sampling depth (m)
Wooley's Buoy	2	38.1508 °S, 145.0745 °E	14	7
Werribee	5	37.9876 °S, 144.7153 °E	8.5	4
Sandringham	3	37.9447 °S, 144.9779 °E	10.5	5
Clifton Springs	6	38.1412 °S, 144.5365 °E	11.5	6
midbay NE	9	38.0502 °S, 144.9139 °E	22.5	10
midbay SW	11	38.1548 °S, 144.8278 °E	22.5	10

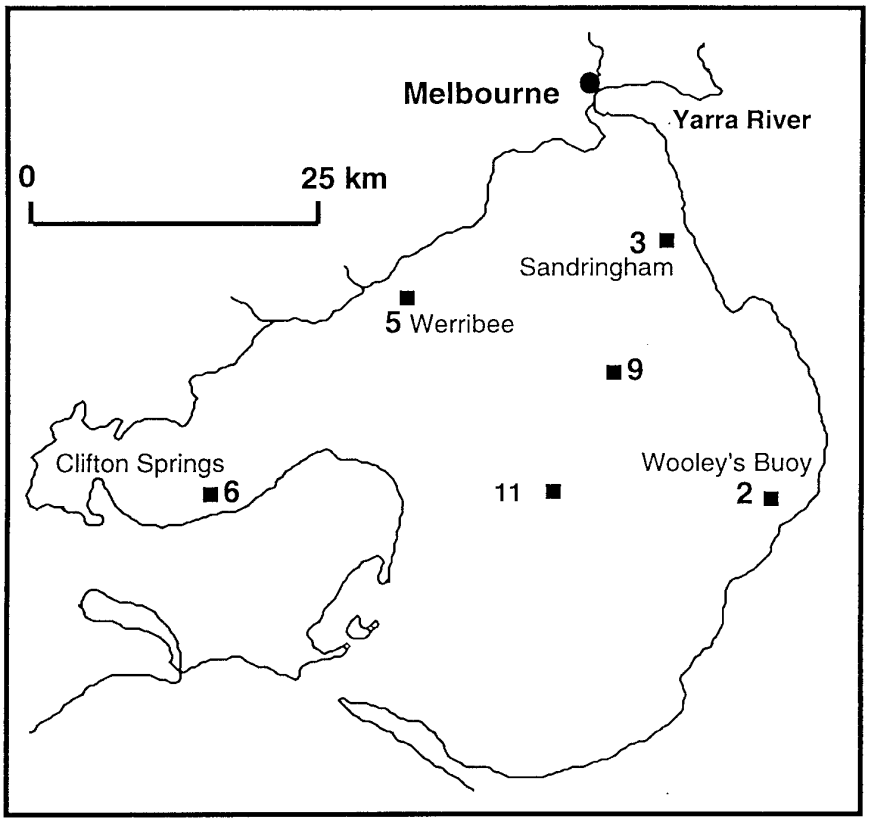


Figure 1. Map showing the locations of the six sampling sites in Port Phillip Bay, Melbourne, Australia. Seawater for microzooplankton experiments was collected from selected coastal and midbay sites at four weekly intervals from October 1994 until mid-September 1995.

2. METHODS

2.1. The Dilution Technique

The Dilution Technique of Landry and Hassett (1982) was employed to estimate community microzooplankton grazing rates in Port Phillip Bay, as recommended by Holloway and Jenkins (1993) in their review of the role of zooplankton feeding in Port Phillip Bay. This technique is the most widely used method for measuring microzooplankton grazing in coastal and open ocean waters (Paranjape 1987, Gifford 1988, Gallegos 1989, Kamiyana 1994, Dagg 1995 and others). The main advantages of the dilution technique are that it 1) provides both grazing and phytoplankton growth estimates in a single experiment and 2) involves minimal handling and disruption of phytoplankton and microzooplankton grazers.

The method is based on the exponential growth of phytoplankton (as estimated by chlorophyll *a* concentration) in a series of diluted seawater samples, which reflect grazer density. The technique uses differences in measured net chlorophyll *a* growth rates at different dilution levels to effectively uncouple growth and grazing rates. The net observed rate of growth at each dilution level is the difference between the instantaneous rate of chlorophyll *a* growth and mortality due to grazing;

$$k = \ln (P_t/P_0)/t = \mu - g$$

where P_t and P_0 are the final and initial chlorophyll *a* concentrations at times t and 0, and k and g are the instantaneous coefficients of growth and grazing, respectively. Grazing and growth coefficients are determined from a linear least squares regression analysis between apparent growth rate of chlorophyll *a* and the proportion of undiluted seawater in each bottle. The intercept is k and the slope is g (see Figure 2 for Dilution Method plots). The significance of the regression line is tested at a level of $p<0.05$ and 95% confidence intervals are calculated for the coefficients of growth (k) and grazing (g).

In addition to exponential growth, the dilution method also assumes that specific phytoplankton growth rates will be density-independent in a dilution series but that grazing mortality will be dependent on the grazer density, as reduced by dilution. It also assumes that growth of phytoplankton is not limited by available or regenerated nutrients. For oligotrophic waters, addition of dissolved inorganic nitrogen to experimental seawater is necessary to ensure exponential growth during the incubation period.

Other requirements of the method include phytoplankton concentrations in the dilution series which are neither at “threshold” nor at “saturated” feeding levels for the microzooplankton. These and other assumptions, however, may be difficult to test. The advantages and disadvantages of employing the dilution technique and the assumptions of the method are further detailed in Landry (1993).

2.2. Field Collections

From October 1994 to early March 1995, seawater was collected during daylight hours from approximately mid-depth using a water pump and polycarbonate tubing from onboard the

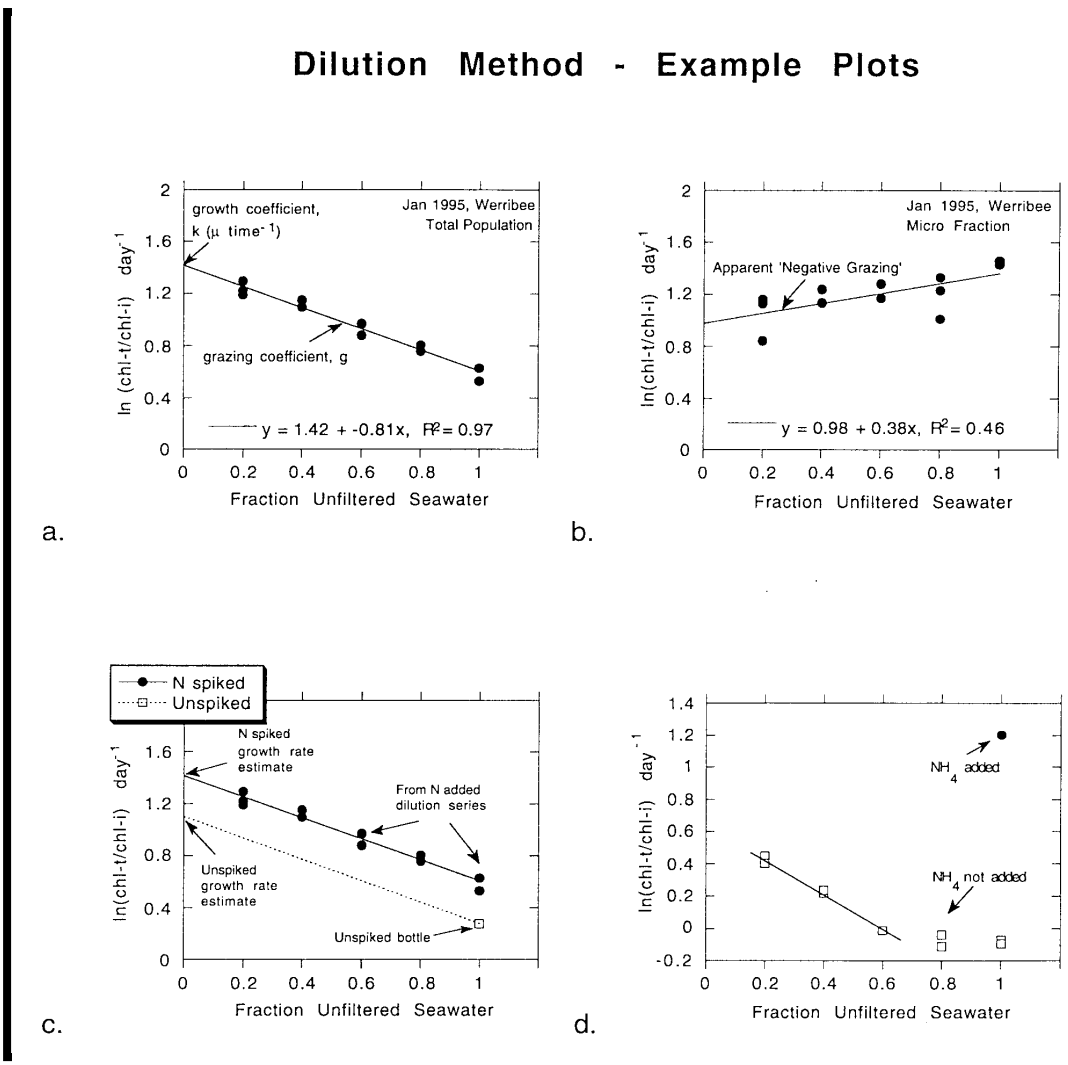


Figure 2. Examples of dilution method plots for estimating chlorophyll *a* growth rate and microzooplankton grazing rate:

a) common plot type: X-axis represents the fraction of unfiltered seawater; Y-axis represents apparent growth rate; Y-intercept (k) is the estimate of chlorophyll *a* growth rate in the absence of grazers; the negative slope (g) is the estimate of grazing rate.

b) plot in which the slope of the regression is positive; the grazing rate is therefore "negative" suggesting that grazing rate increases as the fraction of unfiltered seawater and density of grazers, decreases. Such plots were generally found for the micro fraction only. In most cases where g<0, g was not significantly different from 0.

c) plot which shows the estimation of chlorophyll *a* growth rates for nonspiked treatments. The slope (g) for the NH_4 spiked dilution series is applied to the net growth rate in the nonspiked undiluted treatment (open square, X=1).

d) plot which shows data from an experiment in which nitrogen was not added to the dilution series. At X= 0.8 and 1.0, nutrient limitation is apparent and grazing exceeds algal growth. An undiluted seawater treatment spiked with NH_4 (solid circle, X=1.0) is included for comparison and shows nutrient limitation in all nonspiked treatments.

Marine Science Laboratory's research vessel, the *Melita*. The seawater from each site was stored in two acid washed and rinsed 20-L polycarbonate carboys. To maintain near ambient light and temperature conditions during storage on the *Melita*, the carboys were housed in a large seawater-filled tank, fitted with a shadecloth cover (permitting about 30% ambient light) and entry and exit ports to allow for the continuous pumping of surface seawater. From late March through to mid-September, experimental seawater was collected using a 5-L Niskin water sampler from onboard a Monash University research vessel, stored in carboys and transported immediately to the laboratory at the Water Studies Centre, Monash University. All experiments were conducted at the Water Studies Centre and were initiated within 12 hr of sample collection.

2.3. Experimental Set-up

Prior to each experiment, all incubation bottles (2.4 L, polycarbonate), carboys (20 L, polycarbonate) and other glass and plastic labware were detergent (phosphorus free) washed, acid (10% HCl) washed and thoroughly rinsed with Milli-Q water. Filtered seawater from each site was prepared from the contents of one of the two carboys by filtering seawater through an acid-washed and rinsed 0.2 µm, 147 mm diameter membrane filter using an *S&S* filtering system. The filtered seawater was used to dilute the seawater contained in the remaining carboy. The filters were frozen in liquid nitrogen and later extracted for calibration purposes.

In order to minimise damage to large phytoplankton cells and fragile protozoan grazers, the experimental water was not pre-screened through a 200 µm mesh for the elimination of net zooplankton. Experimental water therefore contained some mesozooplankters (eg. copepods and large heterotrophic dinoflagellates) in the size range of 200-2000 µM.

The dilution series used in each experiment generally comprised treatments of 20, 40, 60, 80 and 100% unfiltered seawater. Each 2.4 L incubation bottle was gently filled with water using silicone tubing (previously rinsed) which was positioned in the bottom corner of the bottle in order to avoid bubbling and potential damage to phytoplankton cells and grazers. All dilution levels were run in duplicate and, on occasion, triplicate.

Due to low dissolved inorganic nitrogen conditions which prevail in Port Phillip Bay waters (Longmore *et al.* 1995), and the need to avoid nutrient limitation during the 24 hr period of incubation, inorganic nitrogen (either as NO_3 or NH_4) was added to all incubation bottles, except for those run as controls. The amount added was dependent on initial standing stocks of chlorophyll *a* and projected requirements for growth. Approximately 1-3 µM NO_3 was added to experimental bottles in October, 3µM NH_4 was added in November and thereafter, all experimental bottles received 1.5 - 2.0 µM NH_4 prior to incubation.

In most experiments, a control bottle containing 100% unfiltered seawater and no additional nitrogen was also incubated (Table 2). The observed growth rate from this bottle was added to the grazing rate determined for the nitrogen-spiked dilution series in order to estimate the growth coefficient for phytoplankton in the absence of additional nutrient (see Figure 2c).

Table 2. Microzooplankton grazing experiments which included control bottles.

Station	No.	Experiments which included control bottles (100% unfiltered seawater, nitrogen not added)
Wooley's Buoy	2	all except October and November 1994
Werribee	5	all except October 1994
Sandringham	3	all except October 1994
Clifton Springs	6	all except October 1994
midbay NE	9	all except January 1994
midbay SW	11	all except October 1994

An additional bottle, containing 100% filtered seawater and additional nitrogen, was also incubated and used as a check on the efficiency of filtration and the extent of growth in the presence of phytoplankton cell contamination.

All bottles were incubated in a water-filled incubator with a clear acrylic base and maintained at near ambient Bay temperatures by circulating the bath water through a heating/cooling unit set to the *in situ* temperature. Ten Duro-lite® True-light® 40W fluorescent tubes, located approximately 20 cm below the incubator, were used as a light source for phytoplankton growth. A metal mesh fixed to the underside of the acrylic base attenuated the light to 90-100 $\mu E\ m^{-2}\ s^{-1}$. The bottles were exposed to a 12hr light: 12hr dark regime during September to March and to a 10 hr light : 14 hr dark regime during April to late August. Bottle incubations began after local sunset, between 1800 hr and 2300 hr, and continued for 24 hr.

2.4. Sample Collection and Analysis

Before and after incubation, 20 ml seawater samples were removed from a set of selected incubation bottles for inorganic nitrogen analysis (NH_4, NO_2, NO_3). Nutrient samples were filtered through either a Whatman GF/F filter or a 0.2 µm polycarbonate membrane to remove particles, and then stored in Whirlpak bags at -20 °C. All nitrogen analyses were conducted at the Marine Science Laboratory in Queenscliff.

Water samples for chlorophyll analysis were removed before and after incubation to establish initial chlorophyll *a* levels and to estimate phytoplankton growth in incubated treatments. Two samples were removed from each bottle; one sample for the measurement of total chlorophyll *a* (>0.2 µm) and one sample which was size-fractioned in order to examine growth and grazing rates for the pico (0.2-2 µm), nano (2-20 µm) and micro (>20 µm) fractions. Fractionation was conducted using a three tiered filtration apparatus, 0.2 µm and 2.0 µm membrane filters (Poretics) and filters made from 20 µm nylon mesh (Nitex). Following filtration under low vacuum, the filters were immediately frozen in liquid nitrogen for short-term storage. Chlorophyll *a* was extracted overnight in 90% acetone at 4°C in the dark and measured in a fluorescence spectrophotometer (Hitachi F-2000). Concentration of chlorophyll *a* was calculated using the equations of Jeffrey and Humphrey (1975) and calibrations were conducted using acetone extracts of algal cells collected on the large 0.2 µm membrane filters, which were used to prepare filtered seawater.

Additional water samples (100 ml) were removed from the 100% unfiltered seawater treatments, before and after incubation, for microscopic analysis of phytoplankton taxa and cell counts. Samples were preserved using 1% Lugol's Iodine solution followed by 2% buffered formalin (final concentration). Algal cells were identified and enumerated using 25 ml settling chambers and inverted light microscopy. Biovolume was determined using the mean of three measurements (length, diameter and perpendicular diameter) and assigning a geometric shape to each species (Table 3). Most cells could be estimated using a sphere, ellipse, cone, cylinder or elliptical cylinder. Some cells, with more complex shapes, were estimated using the sum of the same or different solids.

Table 3. Cell shapes and representative equations used to calculate biovolume of phytoplankton cells. V= volume, L = length and D = diameter (longest for elliptical solids) and Dp = perpendicular diameter (perpendicular to D).

Algal Cell Shape	Equation for Calculating Biovolume
sphere	$V=3.141\ (4/3)\ (1/2D)^3$
cylinder	$V=3.141\ [(1/2)D]^2\ L$
cylinder (elliptic)	$V=(3.141/4)\ L\ D\ Dp$
ellipsoid	$V=(3.141/6)\ L^2\ D$
cone	$V=31.41(1/3)[(1/2)D]^2\ L$

Zooplankton taxa were collected from seawater remaining in the incubation bottles following each experiment. A PVC cylinder fitted with 20 μm nylon mesh was used to collect grazers from the equivalent of 6-10 L of undiluted seawater. The contents were then backwashed into 60 ml bottles with filtered seawater and preserved with 2-3% buffered formalin (final concentration). Counts of fixed heterotrophic protozoans were conducted using inverted light microscopy; larger grazers were identified and enumerated using stereomicroscopy. Though heterotrophs <20 μm are known to dominate the microzooplankton of coastal waters (Beers *et al.* 1975), their identity was not examined in this study due to the complex preservation techniques required to adequately preserve all non-loricate ciliates and heterotrophic nanoflagellates (Sherr *et al.* 1993, review in Pierce and Turner 1992).

3. RESULTS

3.1. Initial Conditions : Temperature and Nitrogen Concentration

All experimental bottles were incubated at near ambient water temperatures. Figure 3a shows the range (10-22°C) in monthly temperature observed at mid-depth at sites sampled during the 1994-95 study period. Mid-depth water temperatures were generally <15°C during May to November and in the range of 18-22°C during December through to late March (see Appendix 1 for site-specific details).

Initial levels of ammonia, determined for experiments conducted from December 1994 to September 1995, show concentrations of <0.1 - 1 μM NH_4 in seawater samples prior to incubation (Figure 3b). NO_3 and NO_2 levels are not reported as they were always at or near detection levels. Ammonia concentrations of <0.3 μM were commonly found in water collected at mid-depth during the summer months (January to March 1995); levels >0.5 μM NH_4 were observed in December 1994 and during autumn and winter months (April to August 1995). In July 1995, an unusually high NH_4 concentration of 20 μM was observed in mid-depth water collected from Wooley's Buoy. In non-summer months, NH_4 levels were often spatially variable with coastal stations generally showing higher levels than midbay sites (see Appendix 1).

3.2. Initial Chlorophyll Concentrations and Algal Cell Densities

Figure 4 shows mid-depth chlorophyll *a* levels (with size fraction composition) and phytoplankton concentrations (cells >2 μM) for all sites with the exception of Clifton Springs (sampled on only three occasions; data included in Appendices 1 and 2).

Chlorophyll *a* concentrations for the total phytoplankton population were $\leq$1 μg L^{-1} at most sites during the spring and summer months (October 1994 - late March 1995). Over this period, levels at Sandringham tended to be slightly higher, with observations in the range of 0.5-1.5 μg chlorophyll *a* L^{-1}. Chlorophyll *a* concentrations were often much higher during the autumn and winter months (April to September 1995) with concentrations at Werribee and Sandringham generally about 2 μg chl L^{-1}; Wooley's Buoy exhibited autumn and winter levels in the range of 1-5 μg chl L^{-1} while concentrations at the midbay sites were in the range of 1-4 μg chl L^{-1}). Over the sampling period, chlorophyll *a* concentrations were much more variable at Wooley's Buoy and the midbay sites than at Sandringham and Werribee.

During the spring and summer months, levels of chlorophyll *a* within the micro fraction were at times very low (<0.1 μg L^{-1}), comprising <20% of the total chlorophyll *a*. Inorganic nitrogen levels were also low during this period (Figure 3b). Considerably higher levels of chlorophyll *a* were found in the micro fraction during the autumn and winter months when ambient NH_4 concentrations were typically >0.5 μM. The most notable feature regarding the nano fraction was its dominance (70%) and concentration (2.6 μg chl L^{-1}) at midbay Stn 9 in July. Overall, nano fraction concentrations were generally in the range of 0.2-1 μg chl L^{-1} with dominance appearing primarily during spring (October to December). For all stations,

Initial Temperature and Nutrient Conditions

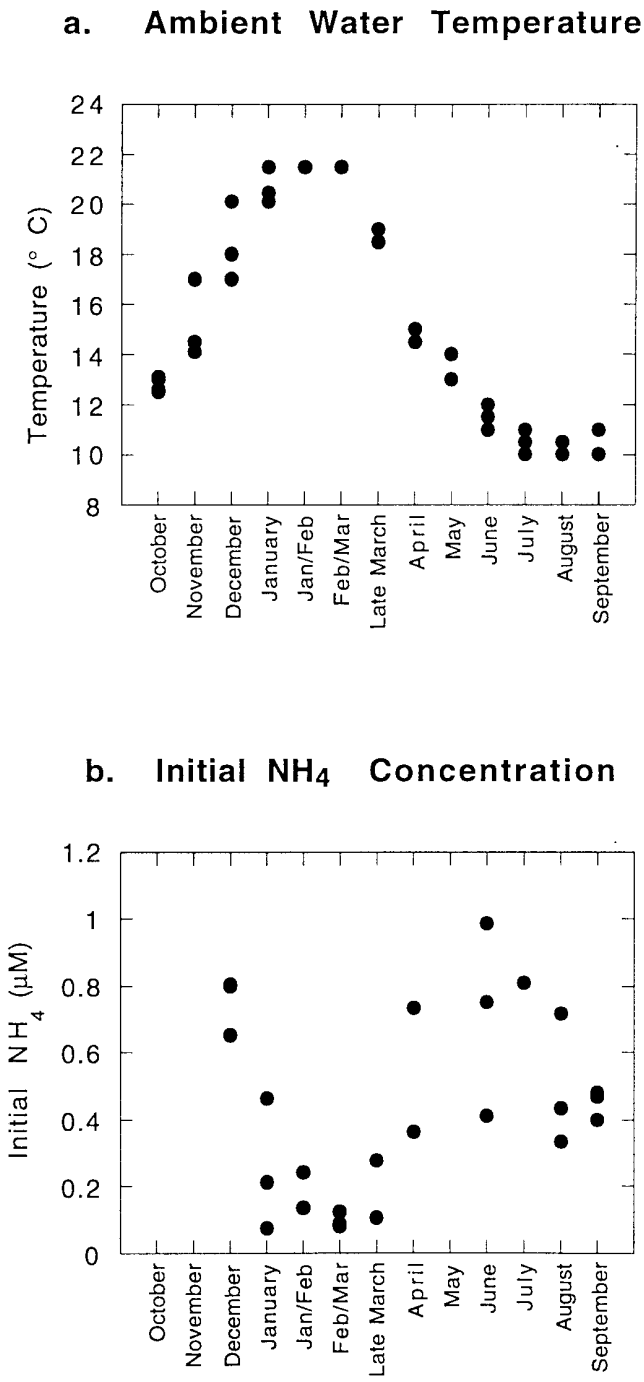


Figure 3. Plots of a) ambient water temperature and b) initial NH_4 concentration in seawater collected from mid-depth at all sites sampled during the study period. Panel b excludes one unusually high concentration of 20 µM NH_4 observed at Wooley's Buoy (Stn 2) in July 1995. See Appendix 1 for data from individual sites.

the pico fraction often represented >40% of the total phytoplankton but in most cases, such dominance occurred when total chlorophyll levels were <1.5 μg L^{-1}.

Phytoplankton cell counts (cells >2 μM only) were also seasonally more variable at Wooley's Buoy and at the midbay sites than at Sandringham or Werribee (Figure 4e-h). For most of the study, cell concentrations fell in the range of $1x10^5$ - $1x10^6$. Peaks were generally observed during the autumn and winter months when chlorophyll *a* concentrations in the combined nano and micro fractions exceeded 1 μg L^{-1}.

3.3. Phytoplankton Composition

Table 4 presents a summary of those species which were seasonally dominant in experimental water collected from mid-depth. Data on species concentration (Appendix 3) usually indicated no major changes in the dominance of phytoplankton taxa following a 24 hr incubation with additional nitrogen.

Table 4. Summary of the dominant phytoplankton species (cells >2 μM) by season.

Season 1994-95	Dominant Diatom Species	Other Dominant Phytoplankton
Spring	*Thallasiosira mala* *Pseudonitzchia pungens*	*Leucocryptus marina* *Katodinium rotundatum* *Pyramimonas sp*
Summer	*Pseudonitzchia pungens* *Rhizosolenia delicatula* *Skeletonema costatum* *Proboscia alata*	*Leucocryptus marina* *Plagioselmis prolonga*
Autumn	*Chaeotoceros socialis* *Pseudonitzchia pungens* *Skeletonema costatum* *Rhizosolenia delicatula*	*Plagioselmis prolonga* *Phaeocystis pouchettii*
Winter	*Pseudonitzchia spp.* *Rhizosolenia spp* *Thalassionema sp* *Skeletonema costatum*	*Gymnodinium spp.* *Plagioselmis prolonga* *Teleaulax acuta*

3.4. Algal Cell Growth Response to Added Nitrogen

In most experiments, the undiluted treatment with additional nitrogen showed an increase in phytoplankton cell concentration which was generally accompanied by an increase in total cell volume (Figure 5a, Appendix 2). Growth rates in cell number and cell volume were variable but generally indicated a net growth rate of ≤ 1 μ d^{-1} or ≤1.5 cell doublings per day.

Chlorophyll *a* and cell count data indicated an increase in mean chlorophyll *a* content per cell (cells >2 μM diam) in most experiments following a 24 hr incubation with additional nitrogen (Figure 5b). In a few experiments conducted during summer, the average post-incubation chlorophyll *a* content per cell was 6-9 times higher than the initial level. When it was more

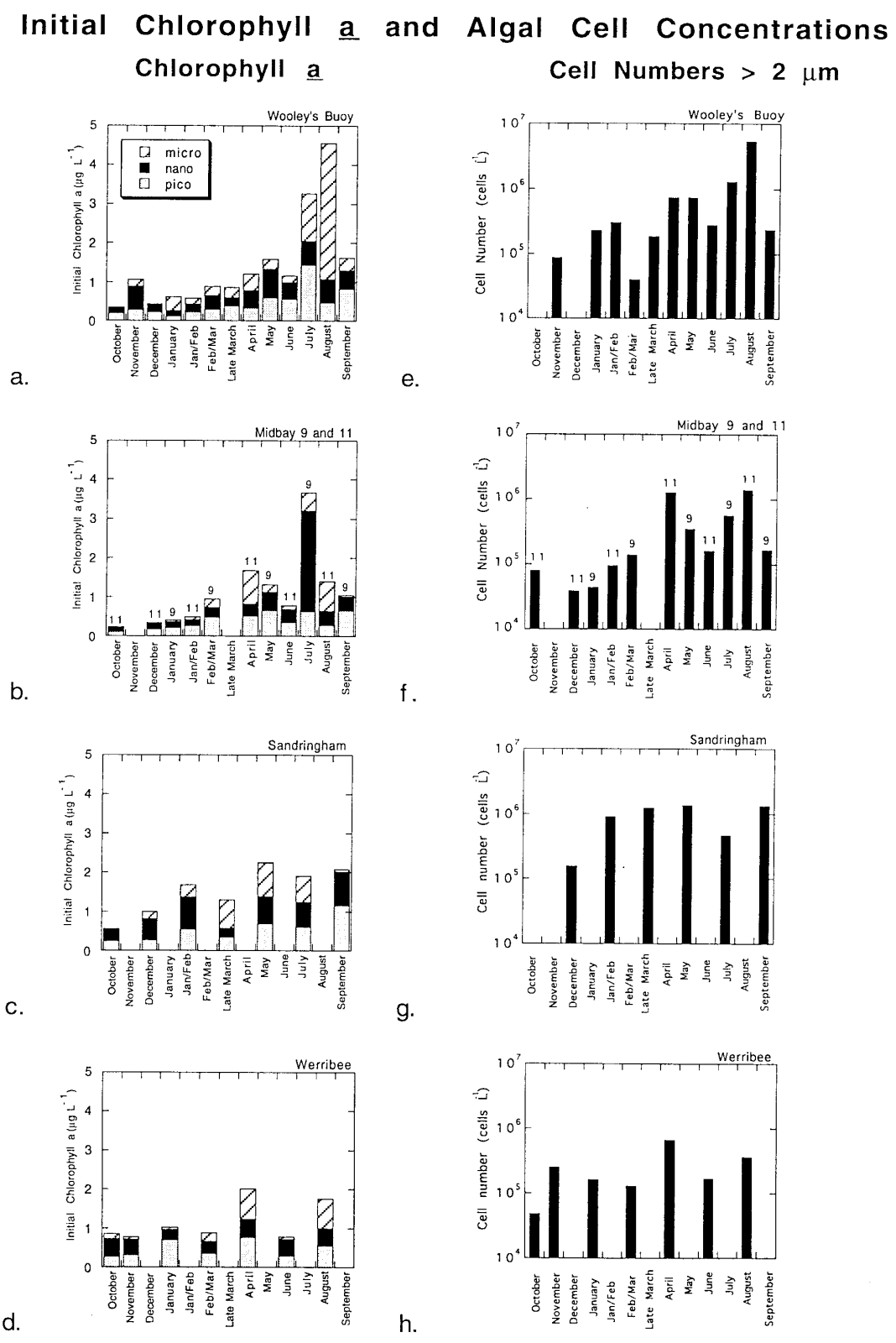


Figure 4. Initial concentrations of chlorophyll *a* (μg L^{-1}) and algal cells (# L^{-1}) prior to incubation. Data includes all sites sampled from October 1994 until mid-September 1995 with the exception of Clifton Springs (Stn 6). Numbers above bars in plots b and f are midbay station numbers. See Appendix 1 and 2 for greater detail.

than 3 times the initial level, total cell numbers following incubation were either equal to or lower than initial cell counts. In some of these cases, the micro fraction (>20 μm) contained a greater proportion of the total chlorophyll *a* following incubation and, in part, explains the observed increases in average chlorophyll *a* content per cell. It should be remembered, however, that when the cellular levels of chlorophyll *a* are elevated without an increase in cell size, phytoplankton growth rates determined using the Dilution Method will be overestimated.

Table 5 summarises the spatial variation in percent change in chlorophyll *a* content per cell and includes experiments conducted from October 1994 until mid-September 1995. Interestingly, the midbay sites exhibited a narrower range and a lower mean than the coastal sites (Stns 2, 3, 5 and 6). The coastal sites showed on average a >100% increase in chlorophyll *a* content per cell following incubation while the midbay sites showed a mean increase of only 44% and a much lower standard deviation than for coastal waters.

Table 5. Percent change in chlorophyll *a* content per cell (nano plus micro fractions) by sampling location. Data includes all seasons.

Stn	Location	% change in chlorophyll *a* content per cell			
		Range	Mean	StDev	N
2	Wooley's Buoy	-39 to 902	120	296	9
3	Sandringham	-25 to 623	230	271	6
5, 6	Werribee and Clifton Springs	-86 to 504	127	210	6
9, 11	midbay Sites	-49 to 140	44	65	10

Table 6. Percent change in chlorophyll *a* content per cell (nano plus micro fractions) by season. Data includes all sampling locations. * data for December only.

Season	1994-95	Temp °C Range	NH_4 μM Range	% change in chlorophyll *a* content per cell			
				Range	Mean	StDev	N
Spring	Oct - Dec	12-18°C	0.6-0.8*	55 to 423	182	151	5
Summer	Jan - Mar	18-22°C	0.1-0.4	-28 to 623	191	238	9
Autumn	April - June	11-15°C	0.4-1.0	-49 to 124	23	54	8
Winter	July - Sept	10-11°C	0.3-0.8	-86 to 902	94	305	9

Seasonal trends are shown in Table 6. The months selected as representative of each season during 1994-1995 were based on patterns observed in ambient water temperature and ammonia concentrations (Figure 3). Experiments conducted during the autumn months showed a narrower range and a lower mean in percent change in chlorophyll *a* content per cell. During this period (April to June), initial NH_4 levels were relatively high (0.4 - 1 μM) at all stations. The autumn data represents five experiments from coastal sites and three from midbay sites.

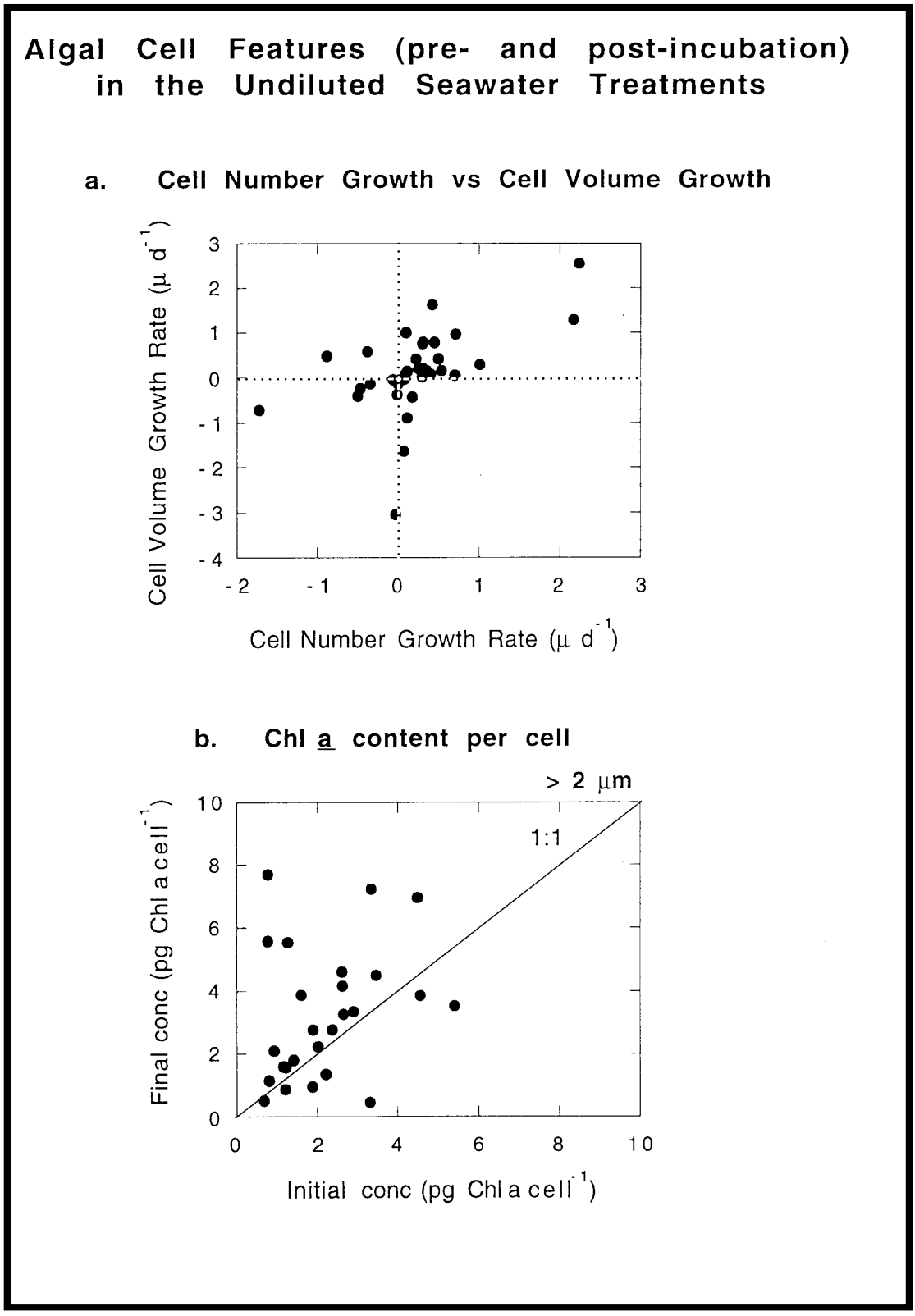


Figure 5. Phytoplankton cell growth in nitrogen-spiked, undiluted seawater treatments. Data represent algal cells >2 μM as cell counts did not include pico-phytoplankton. Plot a shows the relationship between apparent growth rates in cell number and cell volume. See Appendix 2 for site-specific detail. Plot b shows initial vs final concentrations of chlorophyll *a* cell^{-1}.

3.5. Net NH_4 Consumption and Chlorophyll Production

In undiluted treatments, a positive relationship was observed between net losses in NH_4 and net increases in chlorophyll *a* (Figure 6a). In four experiments, slightly higher NH_4 levels were observed following incubation; in these cases, which were conducted during winter (10-12°C), there was little net increase in chlorophyll *a*. At warmer temperatures (>14°C), net increases in chlorophyll *a* were higher and in the range of 1-2.5 µg chlorophyll *a* / µM NH_4 consumed (plot b). High net increases in chlorophyll *a* per µM NH_4 consumed were also associated with low initial NH_4 levels (<0.4 µM) (plot c), which appear primarily during summer. The relationship between chlorophyll *a* growth and NH_4 consumption does not appear to be related to the initial concentration of chlorophyll *a* (plot d).

3.6. Chlorophyll Growth Rates vs Temperature

An examination of the effect of incubation temperature (range 10-22°C) on the chlorophyll *a* growth rates for nitrogen-spiked and nonspiked treatments is shown in Figure 7. Growth rate was positively correlated with temperature for the total population and for all fractions in treatments receiving additional nitrogen. For nonspiked treatments, a weak positive relationship was exhibited by both the total population and the pico fraction; for the nano and micro fractions, the slope was not significantly different from zero (Table 7). Growth rates for nonspiked treatments were similar to treatments with nitrogen spiked growth only when incubation temperatures were low (10-12°C).

Table 7. Statistical analyses of nitrogen-spiked growth rates and nonspiked growth rates vs temperature for the total phytoplankton population and for each size fraction.

Fraction	Nutrient Addition	Slope	Significance	R^2	N
Total	+ NH_4	0.134	p<0.001	0.71	41
Pico	+ NH_4	0.131	p<0.001	0.64	41
Nano	+ NH_4	0.095	p<0.001	0.41	41
Micro	+ NH_4	0.124	p<0.001	0.50	41
Total	none	0.040	p<0.01	0.21	34
Pico	none	0.036	p<0.01	0.20	34
Nano	none	0.033	p=0.09	0.09	34
Micro	none	0.013	p=0.05	0.01	34

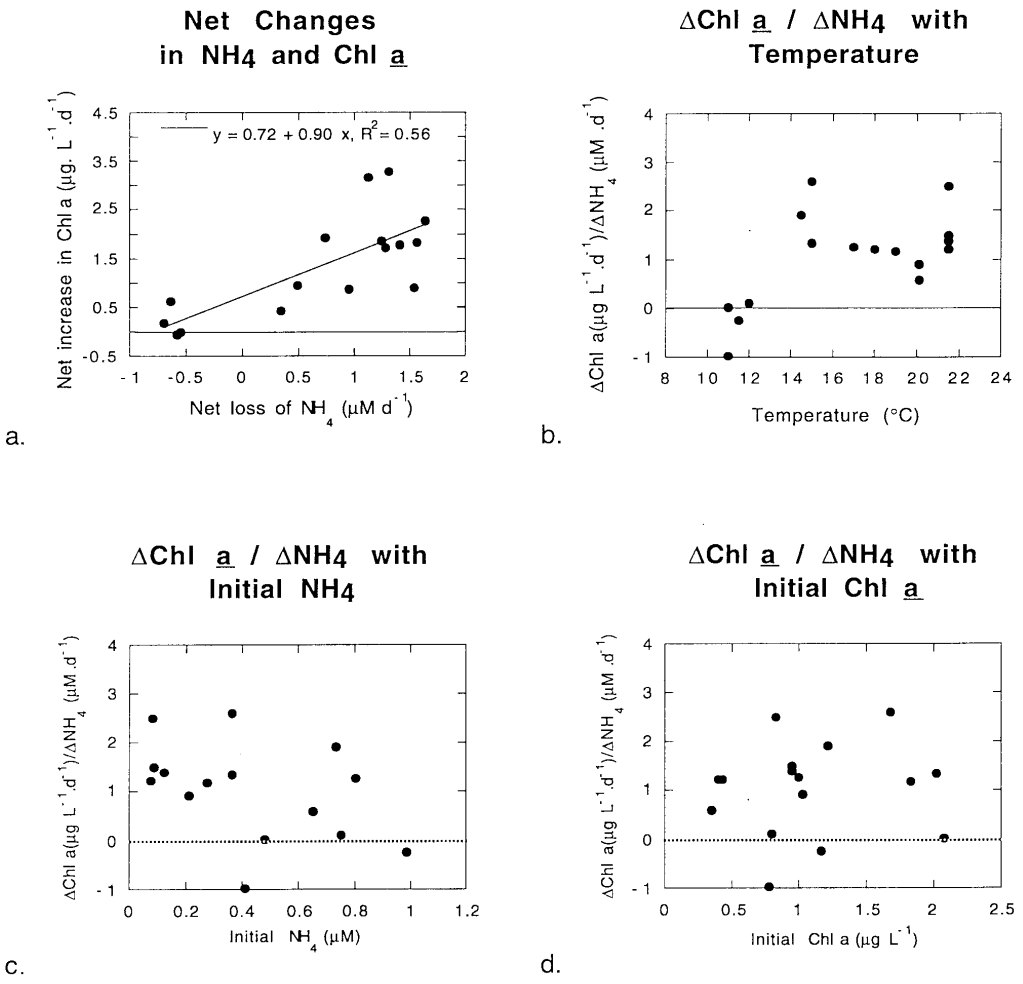


Figure 6. Net changes in NH_4 and chlorophyll *a* concentrations in nitrogen-spiked undiluted seawater and the effects of water temperature and initial levels of NH_4 and chlorophyll *a*. Plot a shows a positive relationship between net increase in chlorophyll *a* concentration and net loss of NH_4. Plots b-d show ΔChl *a* / Δ NH_4 with water temperature, initial ammonia concentration, and initial chlorophyll *a* levels. Data includes values from December 1994 until mid-September 1995.

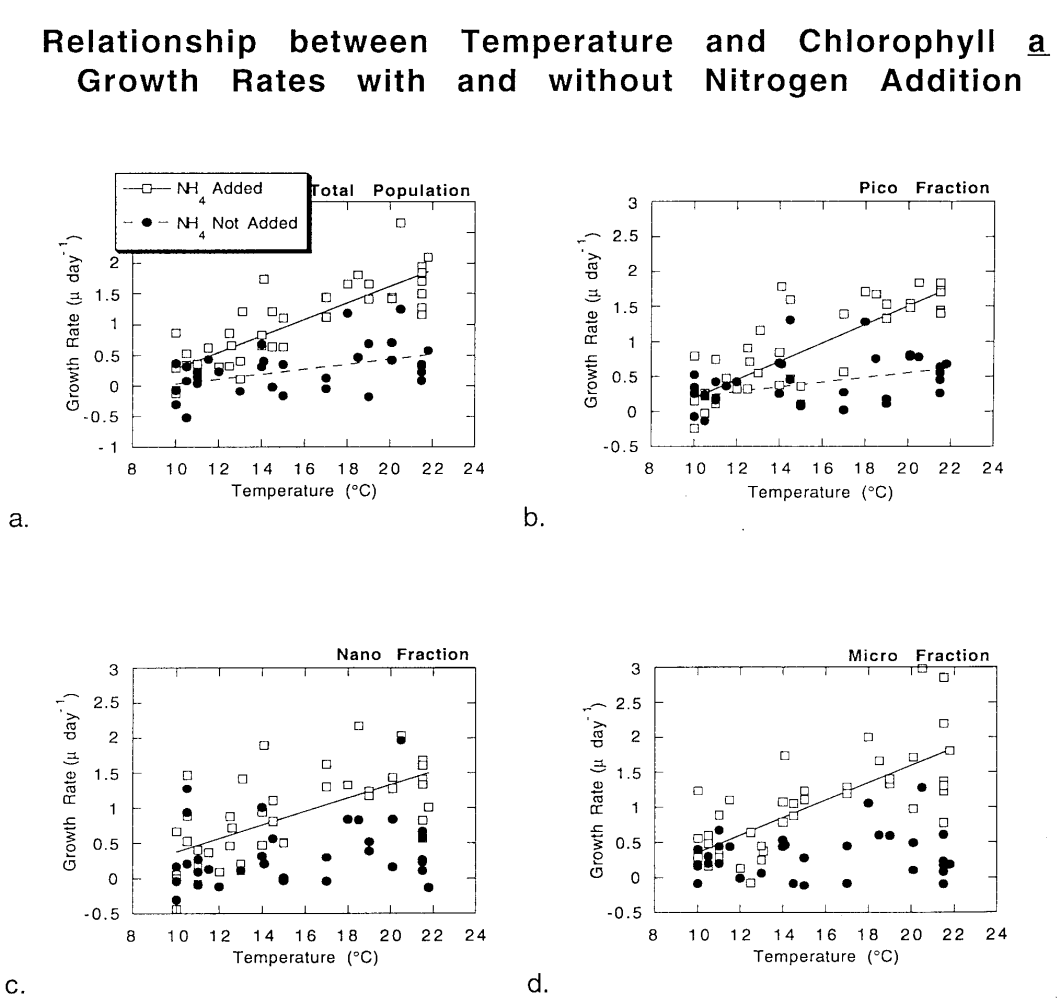


Figure 7. Relationship between temperature and chlorophyll *a* growth rates for the a) total phytoplankton population, b) pico fraction, c) nano fraction and d) micro fraction. Open squares and solid lines represent NH_4 spiked treatments; solid circles and dashed lines represent treatments without nitrogen addition. Least squares linear regression lines are included where the slopes are significantly different from zero. See Table 7 for statistical significance.

3.7. Zooplankton Taxa and Abundance

3.7.1. Taxonomic composition

The zooplankton taxa identified in the undiluted seawater samples (6-10 L) collected from all experiments (n=41) are listed in Table 8. Though formalin is an adequate and commonly used fixative for most zooplankton, it will not preserve the naked or non-loricate protozoans, which require more complex fixation methods (Sherr *et al.* 1993). The zooplankton species list therefore includes only those protists (primarily ciliates) which preserve well in formalin and which were larger than 20 μm. As the net zooplankton were not removed from the experimental water, grazers in this study also include a few taxa >200 μM.

The most common protozoan grazers found throughout the study period were the large dinoflagellate *Noctiluca scintillans* (100-2000 μm diam), the tintinnids *Favella* sp., *Helicostomella* sp., *Tintinnopsis* sp. and the oligotrich, *Strobilidium* sp.. Many other species of loricate ciliates were identified but appeared less regularly. Copepods, including the copepodite and naupliar forms, appeared in all samples and were largely represented by two *Acartia* species, *Paracalanus indicus* and the cyclopoid, *Oithona similis*. Cladocerans were also common year-round with apparent seasonal changes in species occurrence. The most notable cladoceran was *Penilia avirostris*, which was relatively abundant (5-20 L^{-1}) at most stations during the summer months.

The larvacean, *Oikopleura dioica*, was also found in samples throughout most of study and in concentrations of 1-7 L^{-1} during the spring, summer and autumn months. Their gelatinous houses (>2 mm diam), which collect food particles and are regularly produced and discarded, were often abundant and generally contained large numbers of faecal pellets. It is unknown whether or not larvacean faecal pellets were produced during incubation but it is highly likely that most or all of the oikopleurids in the experimental bottles died prior to incubation as preserved specimens generally appeared in very poor condition. Oikopleurids are highly sensitive to mechanical disturbance and bottle containment and require very gentle handling procedures when collected for experimental studies (Redden, pers. obser.).

Faecal pellets were commonly observed in most of the samples and were in greatest abundance when grazing coefficients were >0.3 d^{-1}. Many samples throughout the study also contained mucous sheets and large amounts of fine amorphous material of unknown identity. It should be noted that the presence of amorphous and gelatinous matter in incubated water would probably have an effect on the efficiency of filtration for the size-fractioned samples and potentially increase error associated with the fraction data.

3.7.2. Zooplankton abundance

Grazer densities (excluding oikopleurids and medusae) for the 41 grazing experiments are summarised in Table 9. Total concentrations represent densities from mid-depth waters and are conservative estimates as aloricate protists and grazers <20 μm were not preserved. Taxa were grouped according to relative dominance and classification.

Table 8. List of zooplankton taxa identified from undiluted seawater samples (6-10 L) in grazing experiments conducted during October 1994 - September 1995.

Occurrence: r (rare, <5 ind L^{-1}), c (common, 5-50 ind L^{-1}), a (abundant, >50 ind L^{-1})

Phylum or SubPhylum	Class or SubClass	Order or SubOrder	Taxa	Abundance
Sarcomastogophora	Phytomastigophorea	Dinoflagellida	*Noctiluca scintillans*	c-a
Ciliophora	Litostomatea	Haptorida	*Didinium* sp.	r
			Monodinium sp	r
	Choreotrichea	Oligotrichida	*Strobilidium* sp.	c
			4 unid spp.	r-c
	Choreotrichea	Choreotrichida	*Codonella* sp.	c
			Codonellopsis sp.	c
			Ptychocylis sp.	r
			Favella sp.	c-a
			Helicostomella sp.	c-a
			Metacylis sp.	r
			Eutintinnus sp.	r
			5 unid tintinnid spp.	c
Arthropoda	Copepoda	Calanoida	*Acartia* spp.(2)	c-a
			Paracalanus indicus	c-a
			Tortanus barbatus	r
		Cyclopoida	*Oithona similis*	c-a
			Oithona rigida	c-a
			Unid cyclopoids	r
		Harpacticoida	Unid harpacticoids	c
	Branchiopoda	Cladocera	*Evadne nordmani*	c
			E. tergestina	c
			Podon intermedius	c
			P. polyphemoides	c
			Penilia avirostris	c-a
Urochordata	Larvacea	Oikopleuridae	*Oikopleura dioica*	r-c
	Juvenile forms and other zooplankters:		Various nauplii	c-a
			Bivalve larvae	c-a
			Gastropod larvae	c
			Echinoderm larvae	c
			Polychaete larvae	r
			Pteropod larvae	r
			Decapod zoea	r
			Unid rotifers	r
			Unid medusae	r

Table 9. Summary of grazer densities (# ind L^{-1}) in the undiluted experimental bottles. Counts of larvaceans and medusa not included. All grazers collected with 20 µm mesh.

Station	Date 94- 95	*Noctiluca* # L^{-1}	Ciliates # L^{-1}	Nauplii # L^{-1}	Copepods # L^{-1}	Cladocerans # L^{-1}	Other # L^{-1}	Total # L^{-1}
Wooley's	12 Oct	2	2	10	4	0	4	21
Buoy	9 Nov	27	276	12	8	1	2	327
Site # 2	6 Dec	85	30	4	64	1	2	185
	4 Jan	22	2	4	8	6	3	46
	31 Jan	52	9	9	27	11	3	109
	27 Feb	6	82	25	38	7	14	171
	27 Mar	<1	27	<1	1	0	<1	29
	26 Apr	68	19	17	6	1	2	113
	23 May	45	10	9	3	0	1	67
	20 Jun	<1	150	7	5	0	<1	162
	18 Jul	18	8	6	1	8	2	44
	15 Aug	46	69	3	2	4	1	125
	11 Sep	14	47	4	2	<1	1	69
Sandringham	13 Oct	49	73	8	6	1	6	142
Site # 3	11 Dec	102	66	5	6	1	33	213
	24 Jan	92	66	26	23	0	7	213
	29 Mar	<1	1137	35	10	0	9	1192
	24 May	19	332	10	3	<1	1	364
	17 Jul	2	23	8	3	<1	1	38
	14 Sep	<1	45	7	8	0	<1	61
Werribee	13 Oct	10	11	13	11	0	5	49
Site # 5	10 Nov	3	56	4	4	0	1	68
	4 Jan	17	4	21	27	5	3	77
	3 Mar	2	40	33	7	<1	4	86
	24 Apr	1	78	23	31	0	13	146
	22 Jun	6	69	7	2	<1	<1	84
	14 Aug	9	82	16	3	<1	2	113
Clifton	14 Oct	3	40	2	5	0	<1	51
Springs	11 Nov	43	42	16	8	<1	6	115
Site # 6	28 Mar	32	4	34	23	<1	18	111
midbay NE	5 Jan	134	5	6	11	20	1	176
Site # 9	1 Mar	11	92	23	35	7	6	173
	22 May	10	36	7	4	1	1	59
	20 Jul	3	13	5	1	<1	1	23
	10 Sep	<1	15	3	8	<1	1	27
midbay SW	14 Oct	3	4	3	9	<1	2	21
Site # 11	10 Dec	35	3	5	22	2	2	68
	2 Feb	39	<1	3	23	2	<1	67
	27 Apr	4	37	3	7	0	2	53
	21 Jun	4	328	8	4	0	<1	344
	19 Aug	27	5	2	3	<1	<1	37

Of particular significance was the year-round occurrence of the large dinoflagellate, *Noctiluca scintillans*, in seawater samples collected from mid-depth at all sites sampled. *Noctiluca* densities, ranged from <1-134 L^{-1}, with concentrations of >50 L^{-1} occurring during the summer months at the eastern Bay stations (Wooley's Buoy, Sandringham and midbay Stn 9).

Tintinnids were present in all experimental water but densities were highly variable (<1 to >1000 L^{-1}). Interestingly, tintinnid concentrations were very low (<10 L^{-1}) during early and late January when both growth and grazing coefficients were high (Figs 8a and 9a). The highest ciliate densities were observed during March to June with the greatest numbers in seawater from the Sandringham site. *Helicostomella* sp. (40-70 μm long) was found at all sites during most of the study (found in 76% of samples) and was typically the most abundant. loricate ciliate during the summer and autumn months. *Favella* sp. (60-100 μm long) was also present year-round and in highest densities during the winter and spring.

Throughout the study, nauplii were present in mid-depth waters at concentrations of <1 to 35 L^{-1} and with peak densities during summer. They were also more abundant in seawater collected from the coastal sites (4-7 m) than from the midbay sites (10 m). Copepods (primarily copepodites) were seasonally abundant with densities of 10-60 L^{-1} during the summer months at all sites. Cladocerans were present in most samples but densities were generally <5 L^{-1} and rarely exceed 10 L^{-1}. Other taxa comprised various benthic larvae and juvenile zooplankters; collectively, they were most abundant during summer at the coastal stations.

Highly variable total zooplankton concentrations (20-1200 ind L^{-1}) observed throughout the study (Table 9) were largely due to seasonally variable tintinnid concentrations. When ciliates are removed from the total population, grazer concentrations are <200 L^{-1}. And, if densities of both ciliates and *Noctiluca* are excluded, concentrations are <100 ind L^{-1} and typically in the range of 10-50 L^{-1}.

3.8. Spatial and Temporal Variation in Grazing and Growth Rates

3.8.1. Chlorophyll *a* growth rates for coastal and midbay sites

Monthly estimates of chlorophyll *a* growth rate, with and without nutrient addition, are shown for the four coastal and two midbay sites in Figure 8 (for site-specific detail and standard errors, see Appendices 4-7). For both coastal and midbay sites, growth rates in the nitrogen-spiked treatments were typically higher than growth rates without nitrogen addition. Though midbay sites often showed chlorophyll *a* growth rates, which were lower than those for the coastal stations, no consistent pattern was apparent. Seasonal trends in growth rates of both coastal and midbay sites were more evident for the nitrogen-spiked treatments, with highest rates in summer and lowest rates in winter for the total phytoplankton population and for most fractions. Seasonal trends are further discussed in Section 3.9.1

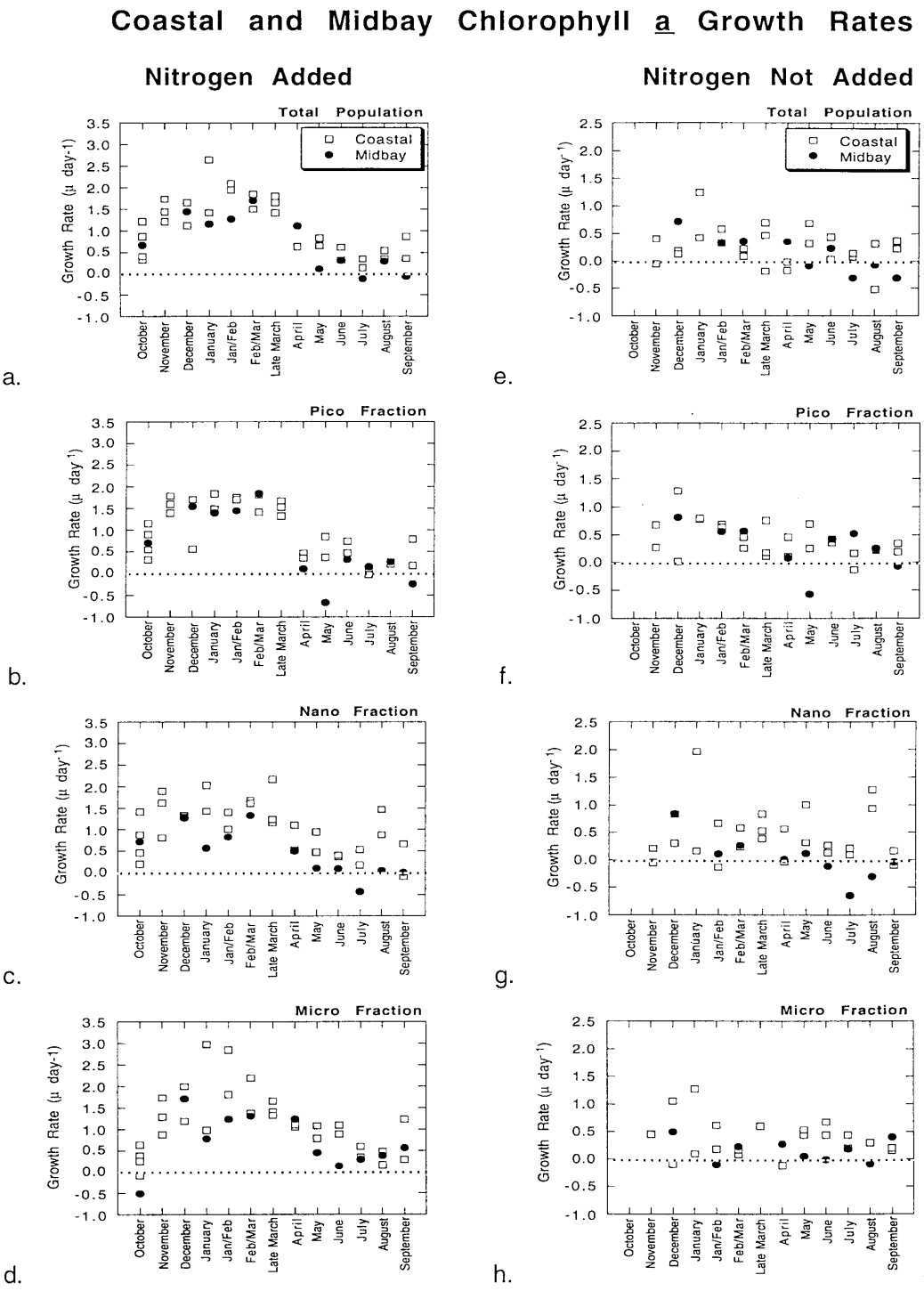


Figure 8. Coastal and midbay chlorophyll *a* growth rate estimates, with nitrogen addition (a-d) and without nitrogen addition (e-f), for the total population and for each fraction, from October 1994 until mid-September 1995. Open squares represent the four coastal stations; closed circles represent the two midbay stations. Dashed lines show k = 0.

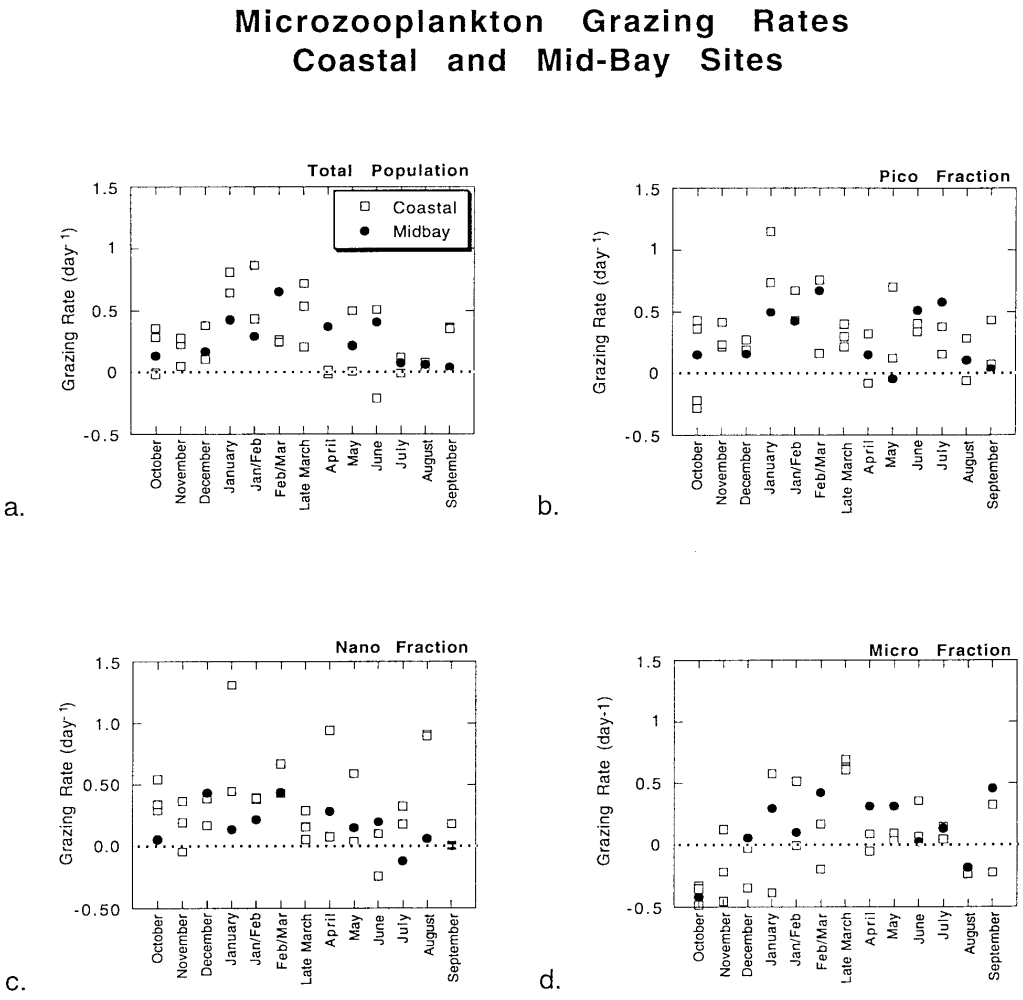


Figure 9. Microzooplankton grazing rates for the a) total phytoplankton population,

b) pico fraction, c) nano fraction and d) micro fraction, from coastal and midbay sites from October 1994 until mid-September 1995. Open squares represent the four coastal stations; closed circles represent the two midbay stations. Dashed lines show g = 0. For more detail of the monthly grazing rates, see Appendices 4-7.

3.8.2. Grazing rates for coastal and midbay sites

Monthly grazing rates for both coastal and midbay sites show seasonal trends in grazing impact for the total phytoplankton population (Fig 9a). Peaks in grazing occurred during the summer months ($g>0.5\ d^{-1}$) with lowest rates observed in winter ($g<0.2\ d^{-1}$). These trends are consistent with seasonal patterns in grazer abundance (Table 9). Monthly grazing rates for the coastal sites do not appear to differ greatly from those of midbay sites. Seasonal trends are detailed in Section 3.9.1. For the pico, nano and microphytoplankton fractions, seasonal trends in grazing (Fig 9b-d) are less apparent than those for the total chlorophyll *a* data.

3.8.3. Limitations of the size fraction data

Interpretation of the fraction data in Figures 8 and 9 is made difficult by the occurrence of a number of negative grazing rate estimates ($g<0$), especially for the micro fraction, which frequently occurred during spring and summer when total chlorophyll *a* concentrations were low ($<1\ \mu g\ L^{-1}$). During this time, the micro fraction generally represented < 20% of the total chlorophyll *a*. Concentrations of chlorophyll *a*, which are at or near levels of detection, may result in errors in growth rate estimates. The fraction data is also subject to error due to potential clogging of the 2 and 20 μm filters in the 3-tiered filtration assembly. The presence of gelatinous and amorphous material, which was found in many of the samples (see Section 3.7.1), would no doubt contribute to filtration inefficiencies.

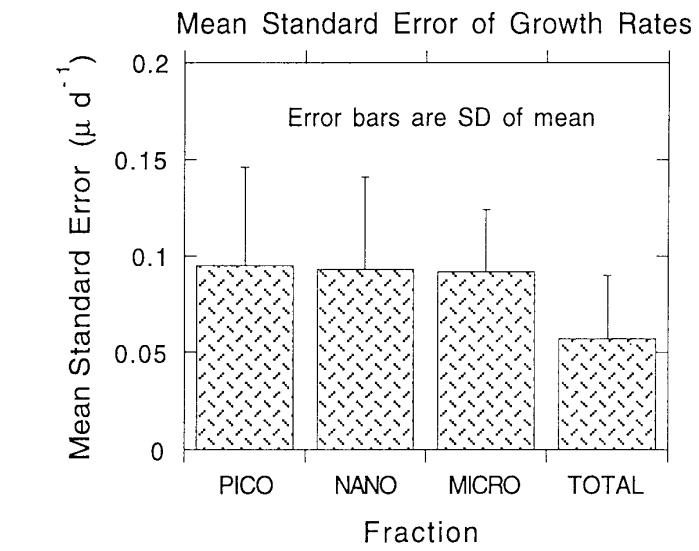
The mean standard errors of the chlorophyll *a* growth and microzooplankton grazing coefficients, for the total phytoplankton population and for each size fraction, for all nitrogen-spiked treatments (N=41), are shown in Figure 10. Mean standard errors associated with both growth and grazing coefficients were much greater for each of the pico, nano and micro size fractions than for the total population. The grazing rates for the individual fractions should therefore be used with due regard of their uncertainty.

3.8.4. Total population: site-specific grazing and growth rates

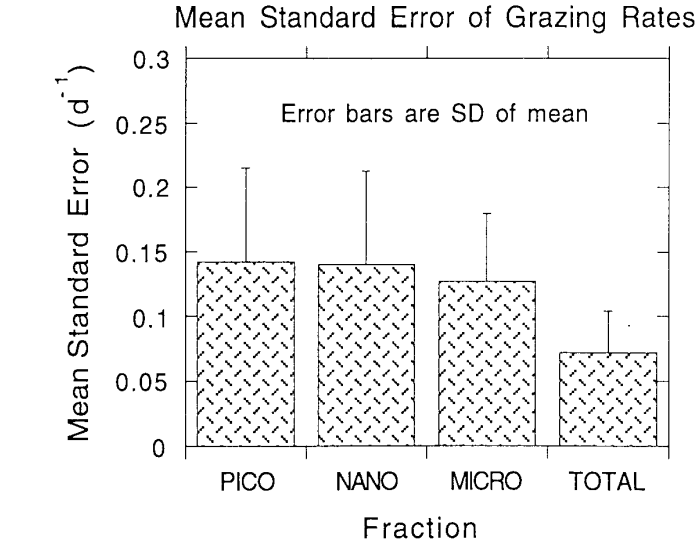
Estimates of monthly grazing and growth rates for the total phytoplankton population from each site sampled (except Clifton Springs) are shown in Figure 11. More detail on the coefficients and standard errors for all stations are included in Appendix 4.

Wooley's Buoy - Total population

Monthly estimates of grazing rate on the total phytoplankton population at Wooley's Buoy (Fig 11a) showed a seasonal pattern with rates of $>0.6\ d^{-1}$ observed in three of the four experiments conducted in summer. A maximum rate of $0.9\ d^{-1}$ occurred in late Jan. Non-summer rates were in the range of $0\text{-}0.5\ d^{-1}$. Grazing rates were very low during the autumn/winter months of April, May, July and August and, on these occasions, were not significantly different from zero.



a.



b.

Figure 10. Mean standard error and standard deviation of the mean for a) chlorophyll *a* growth rates (nitrogen-spiked) and b) microzooplankton grazing rates, for each size fraction and for the total phytoplankton population. Data represent experiments conducted from October 1994 until mid-September 1995 (n=41).

Growth rates of 1-2.5 μ d^{-1} were estimated for nitrogen-spiked treatments in experiments conducted from November to late March; for all other months, growth coefficients were in the range of 0.5-1 μ d^{-1} (Fig 11e). Estimates of growth rate without added nitrogen were >1 μ d^{-1} during early summer (December and January) but were reduced to <0.5 μ d^{-1} during autumn, winter and spring.

Midbay Sites (Stns 9 & 11) - Total population

Grazing rates at the midbay sites (Fig 11b) were generally not as high as those observed at Wooley's Buoy. The highest grazing rate (g = 0.65 d^{-1}) was observed for Stn 9 in early March. For all other experiments, estimates of g were <0.5 d^{-1} and, during the winter/spring period (July to December), the grazing coefficient was <0.2 d^{-1}.

Nitrogen-spiked growth rates for the midbay sites (Fig 11f) showed a similar seasonal pattern to those for Wooley's Buoy. Midbay waters exhibited a growth rate range of 1-1.7 μ d^{-1} during summer and very low rates (0-0.4 μ d^{-1}) during May to September. Nonspiked growth rates approached 0.7 μ d^{-1} during December but were <0.5 μ d^{-1} for all other sampling periods.

Sandringham - Total population

Bimonthly grazing data for Sandringham (Fig 11c) show no clear seasonal pattern or strong similarity to other sites. Grazing rates of 0.4-0.5 were observed in late January, late May and mid-September. All other sampling periods showed rates of <0.2 d^{-1}.

Nitrogen-spiked growth showed rates of about 1 μ d^{-1} in December and peaks of 1.5-2 μ d^{-1} in late January and late March (Fig 11g) . Growth rates were <0.5 μ d^{-1} during July, September and October. Nonspiked growth rates at Sandringham were generally lower than those for Wooley's Buoy and showed rates of ≤0.5 μ d^{-1} during both summer and nonsummer periods.

Werribee - Total population

Grazing rates of 0.3 d^{-1} were determined for three of the four experiments conducted during spring and summer (Fig 11d). A peak rate of 0.8 d^{-1} was exhibited in early January. Grazing impact in autumn and winter at Werribee was very low with rates of <0.1 d^{-1}.

Growth rates for nitrogen-spiked treatments were in the range of 1-2 μ d^{-1} during spring and summer (Fig 11h). This was followed by a decline and growth rates of only 0.3 μ d^{-1} in winter. Nonspiked treatments showed growth rates of <0.5 μ d^{-1} during all months sampled.

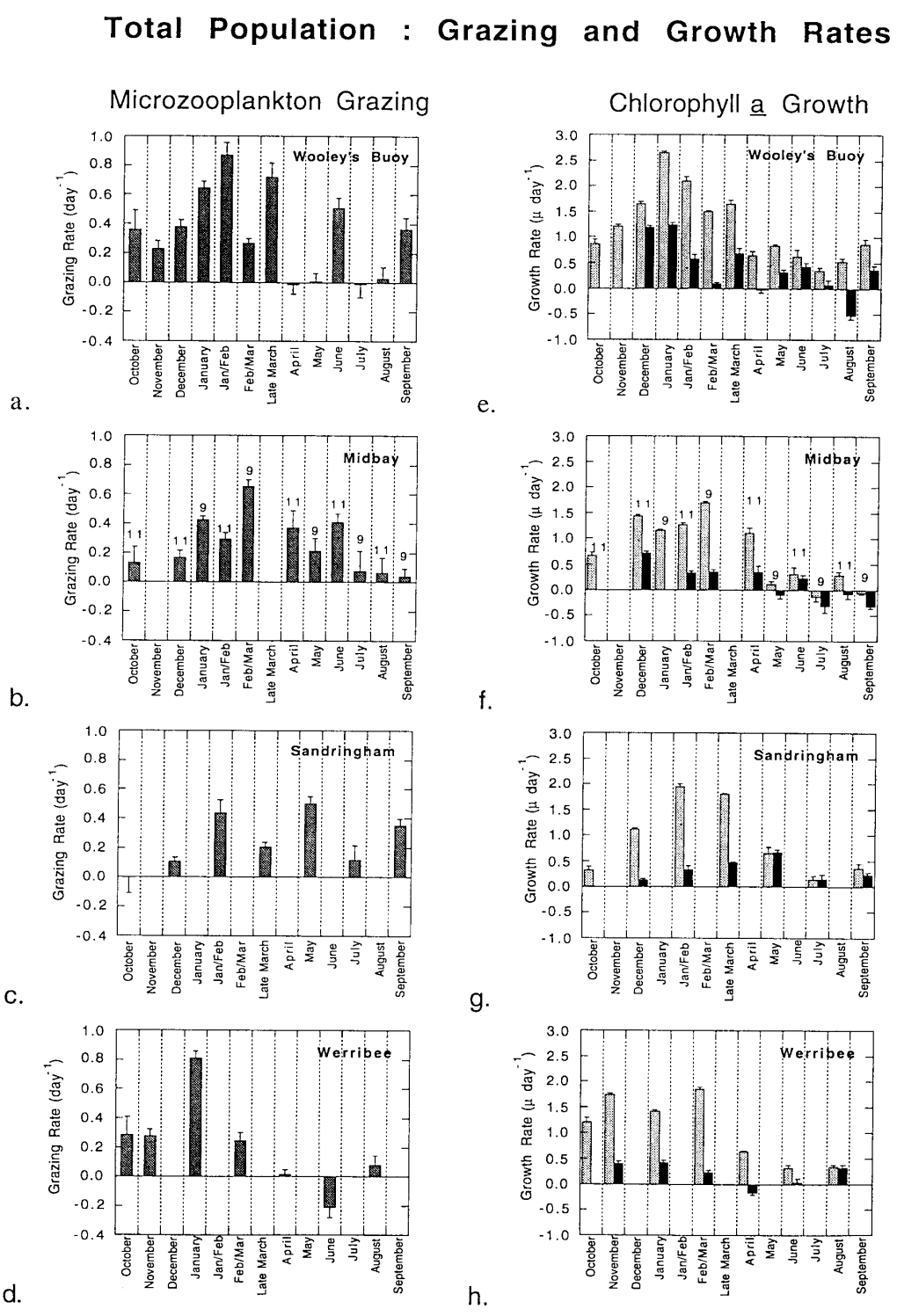


Figure 11. Microzooplankton grazing rates and chlorophyll *a* growth rates for the total phytoplankton population, for sites sampled from October 1994 until mid-September 1995. Plots a-d show grazing rates and standard errors. Plots e-h show chlorophyll *a* growth rates and standard errors: grey bars are nitrogen-spiked treatments; black filled bars are nonspiked treatments (standard error for nonspiked is same as for grazing coefficient). Numbers above bars in plots b and f are midbay site numbers.

3.8.5. Size fractions: site-specific grazing and growth rates

The grazing and growth rates for the three size fractions are shown in Figures 12-14 and in greater detail in Appendices 5-7. Interpretation of the fraction data, however, is somewhat complicated by seasonal differences in the size fraction composition of the phytoplankton and relatively high mean standard errors of the coefficients for grazing and growth (Figure 10, see Section 3.8.3). Analysis of the fraction data is therefore limited to discussion of the general trends.

Wooley's Buoy - Size Fractions

Throughout the study, grazing coefficients for the pico and nano fractions were higher than those for the micro fraction (Fig 12a, 13a, 14a). Grazing rates of >0.5 d^{-1}, on cells >20μm, were found only during early January and late March. The highest grazing coefficients for all three fractions were observed during the summer months.

Pico growth rates in nitrogen-spiked treatments were consistently high (1.3-1.8 $\mu\ d^{-1}$) in experiments conducted during December to late March and were <0.5 $\mu\ d^{-1}$ during the winter months. Though less consistent, the nano and micro fractions also showed peak growth rates in summer (>1.5 $\mu\ d^{-1}$) and reduced growth in winter. In nonspiked treatments, peak growth occurred during summer for all fractions. During winter, growth rates of picos and nanos in nonspiked treatments were often similar to those estimated for treatments with additional nitrogen.

Midbay Sites - Size Fractions

Grazing impact during the summer and winter months appears to be greatest on the pico fraction, with g >0.5 d^{-1} (Figs 13b, 14b). Grazing rates were generally <0.5 d^{-1} for both the nano and micro fractions; no clear seasonal trends were apparent.

All three fractions exhibited peaks in nitrogen-spiked growth rates during summer. Coefficients were highest for the picos, which exhibited growth rates in the range of 1.5-2 $\mu\ d^{-1}$ from December to early March. Summer growth rates for the nano and micro fractions were generally <1.5 $\mu\ d^{-1}$ and during winter, g was <0.5 $\mu\ d^{-1}$ for all three fractions. In non-spiked treatments, growth rates of the pico fraction were higher than those of the nano or micro fractions in both summer and winter.

Sandringham - Size Fractions

Grazing rates for the pico fraction were >0.3 d^{-1} during summer and autumn and <0.2 d^{-1} during winter and spring months. No seasonal trends were apparent for the nano fraction which showed peak rates of about 0.5 d^{-1} in January, May and October. Similar rates were observed for the micro fraction but during summer only.

Seasonal trends in nitrogen-spiked growth rates were shown for all three fractions; growth rates peaked in summer (>1.5 $\mu\ d^{-1}$) and were low in winter (<0.5 $\mu\ d^{-1}$). For treatments

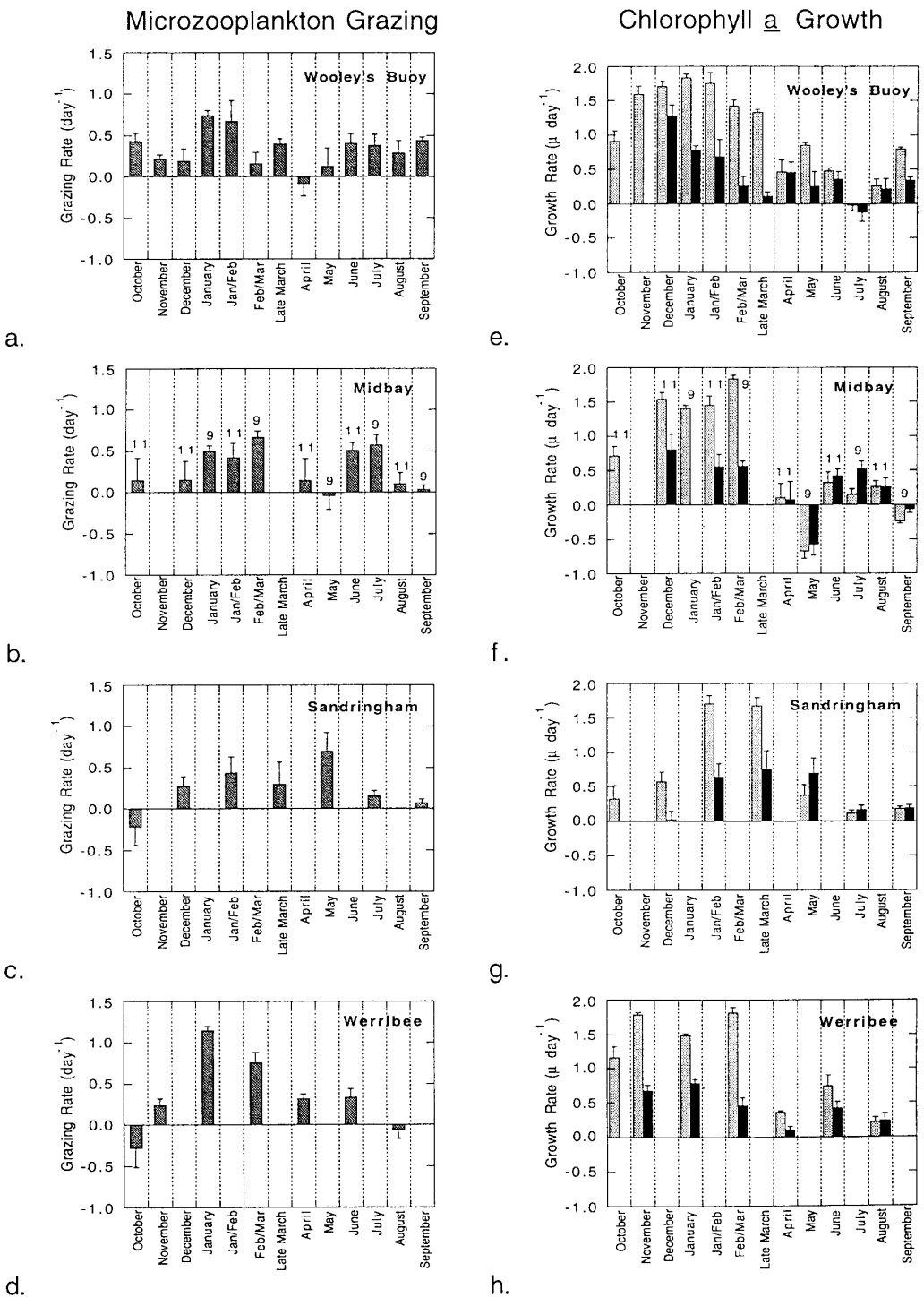


Figure 12. Microzooplankton grazing rates and chlorophyll *a* growth rates for the picophytoplankton fraction, for sites sampled from October 1994 until mid-September 1995. Plots a-d show grazing rates and standard errors. Plots e-h show chlorophyll *a* growth rates and standard errors: grey bars are nitrogen-spiked treatments; black filled bars are nonspiked treatments (standard error for nonspiked is same as for grazing coefficient). Numbers above bars in plots b and f are midbay site numbers.

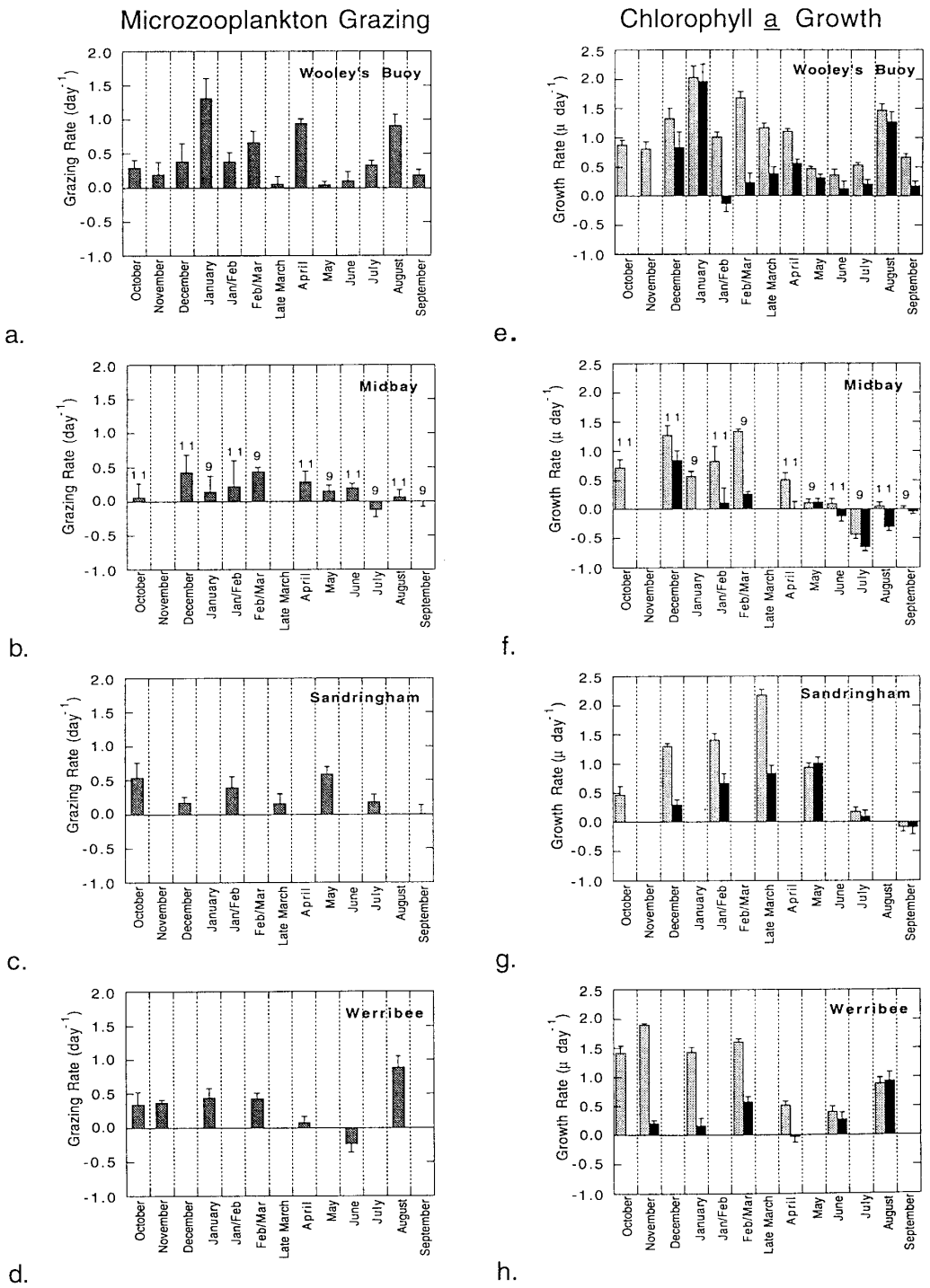


Figure 13. Microzooplankton grazing rates and chlorophyll *a* growth rates for the nanophytoplankton fraction, for sites sampled from October 1994 until mid-September 1995. Plots a-d show grazing rates and standard errors. Plots e-h show chlorophyll *a* growth rates and standard errors: grey bars are nitrogen-spiked treatments; black filled bars are nonspiked treatments (standard error for nonspiked is same as for grazing coefficient). Numbers above bars in plots b and f are midbay site numbers.

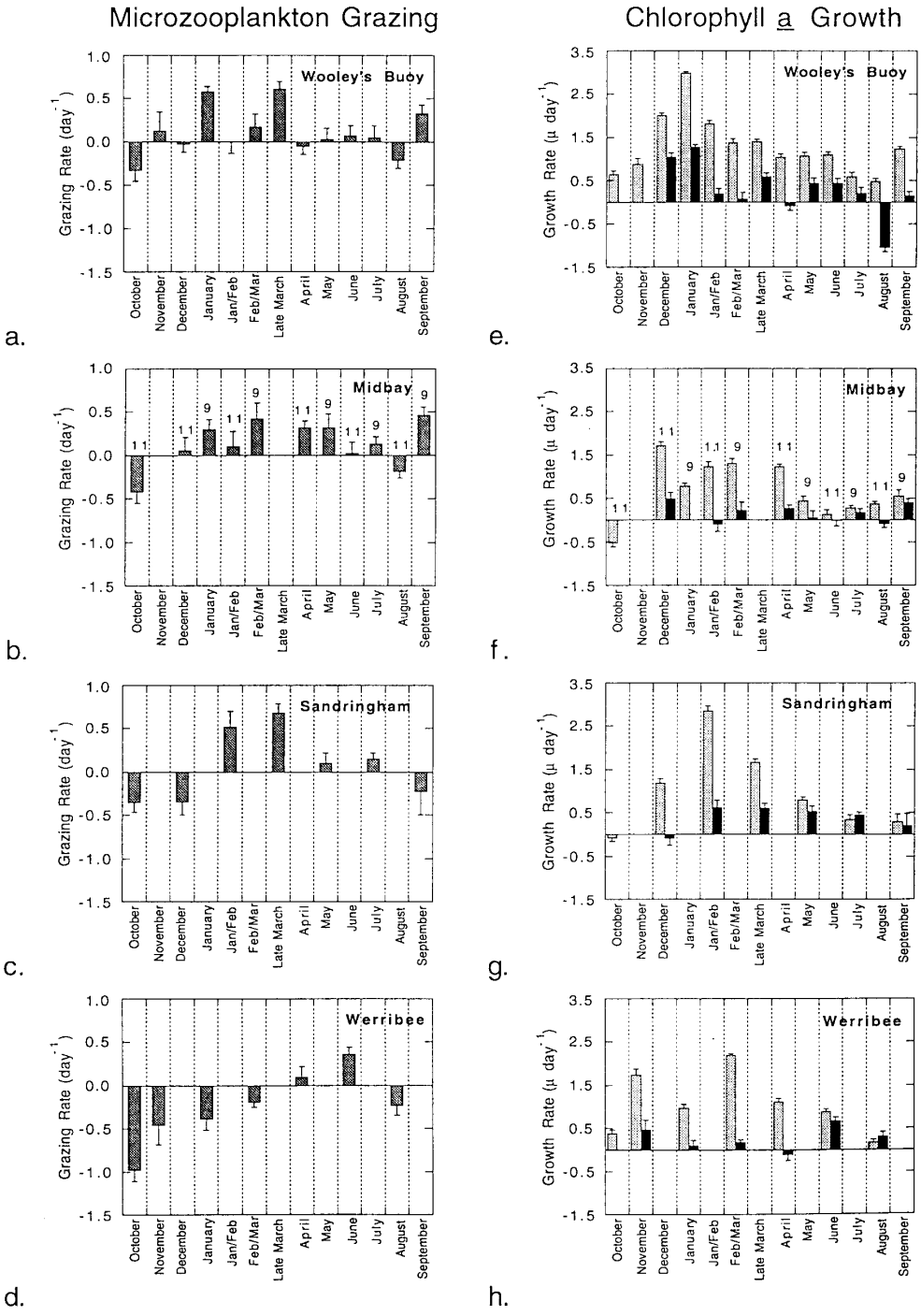


Figure 14. Microzooplankton grazing rates and chlorophyll *a* growth rates for the microphytoplankton fraction, for sites sampled from October 1994 until mid-September 1995. Plots a-d show grazing rates and standard errors. Plots e-h show chlorophyll *a* growth rates and standard errors: grey bars are nitrogen-spiked treatments; black filled bars are nonspiked treatments (standard error for nonspiked is same as for grazing coefficient). Numbers above bars in plots b and f are midbay site numbers.

without nutrient addition, pico and nano growth rate estimates were higher than those for the micro fraction during summer and autumn.

Werribee - Size Fractions

Grazing estimates for the pico fraction show rates of >0.8 d^{-1} during summer and <0.4 d^{-1} for all other periods with little or no apparent grazing on picos in late winter and spring. Grazing rates associated with the nano fraction ranged from only 0.3-0.5 d^{-1} in spring and summer; winter rates were low but a peak of 0.9 d^{-1} was observed in August. The negative grazing rates commonly observed for the micro fraction frequently coincided with very low microphytoplankton abundance (<0.2 μg chl L^{-1}). Overall, there appears to be little or no grazing impact on the micro fraction at Werribee.

Treatments spiked with nitrogen showed growth rates of 1-2 $\mu\ d^{-1}$ for the all fractions during spring and summer months and lower rates ($\leq 1\ \mu\ d^{-1}$) during the autumn and winter months. In treatments without nitrogen addition, the pico fraction exhibited the highest summer growth rates ($>0.5\ \mu\ d^{-1}$). Growth rates for all three fractions during winter were similar to those observed in nitrogen spiked treatments and were as high as $1\ \mu\ d^{-1}$.

3.9. Grazing Impact

3.9.1. Growth vs grazing for nitrogen-spiked and nonspiked treatments

Plots of growth rate vs grazing rate for the total phytoplankton population and for each of the fractions are shown in Figure 15. A 1:1 relationship occurs when growth rate is in balance with grazing rate. In the nitrogen-spiked treatments (plots a-d), growth coefficients were generally higher than grazing coefficients for the total population and for the pico and nano fractions; for the micro fraction, growth rate always exceeded grazing rate. Growth rates in treatments without additional nitrogen (plots e-h) indicate a more balanced relationship between phytoplankton growth and grazing for the total population and for all fractions.

3.9.2. Seasonal and annual estimates of grazing impact

A summary of the seasonal trends (mean ± st dev) in microzooplankton grazing impact on total chlorophyll *a* in nitrogen enhanced and nitrogen limited treatments is presented in Table 10. The following section summarises the seasonal features of both phytoplankton growth and grazing impact on the total phytoplankton.

Spring

Standing stocks of chlorophyll *a* in mid-depth waters during October to December were generally <1 μg L^{-1} at all stations with the midbay sites exhibiting <0.5 μg chl L^{-1}. Nitrogen-

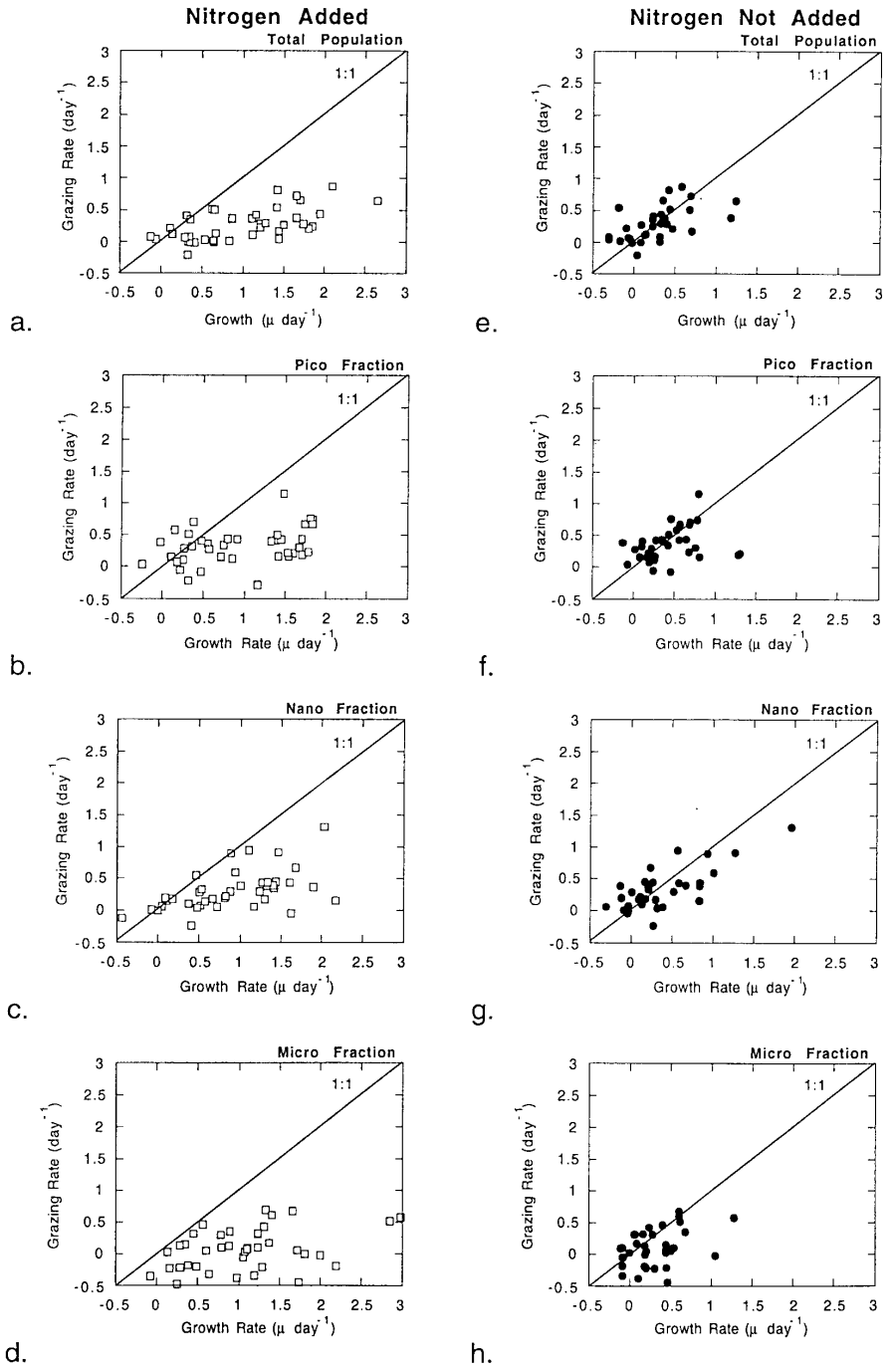


Figure 15. Plots of chlorophyll *a* growth rate versus microzooplankton grazing rate, for the total phytoplankton population and for each fraction. Plots a-d represent treatments spiked with 1-3 µM inorganic nitrogen (n=41). Plots e-h represent treatments without nitrogen addition (n=31). One point excluded from plots b and f as k<-0.5.

spiked chlorophyll *a* growth rates showed an overall mean of 1.1 (± 0.5) μ d^{-1} or 1.6 (± 0.7) chlorophyll *a* doublings per day. Over all sites, potential production for nitrogen enhanced treatments averaged 1.5 μg chl L^{-1} d^{-1}, with lowest production at the midbay sites (0.7 ± 0.6 μg chl L^{-1} d^{-1}) due only to lower initial standing stocks (0.3 ± 0.1 μg chl L^{-1}).

Grazing rates were more variable than chlorophyll *a* growth rates; the lowest mean grazing rate observed during spring was for Sandringham (0.05 d^{-1}) and the highest was for Wooley's Buoy (0.32 d^{-1}). The mean estimate for all sites during spring indicates 16% (± 11%) of the initial chlorophyll *a* stock was grazed, an amount equivalent to 23% (± 17%) of the potential chlorophyll *a* production in nitrogen enhanced waters and 47% (± 34%) in nitrogen limited waters.

Summer

Mid-depth chlorophyll *a* concentrations were slightly higher at all sites during January to March than during spring; the overall summer average was 1 μg chl L^{-1}. Again, the lowest chlorophyll *a* concentrations were found at the midbay sites (mean = 0.6 μg chl L^{-1}). Nitrogen spiked chlorophyll *a* growth rates were higher during summer than all other seasons, with an overall mean rate of 1.7 (± 0.4) μ d^{-1}, representing 2-3 doublings of chlorophyll *a* per day. Estimates of potential production for treatments with additional nitrogen show summer means ranging from 2.1 μg chl L^{-1} d^{-1} (midbay sites) to 8.4 μg chl L^{-1} d^{-1} (Sandringham) with an overall summer average of 4.6 (± 2.7) μg chl L^{-1} d^{-1}.

The highest grazing rates in this study were observed during summer with station means ranging from 0.32-0.63 d^{-1}. When all sites are combined, estimates indicate 38% (± 14%) of the initial chlorophyll *a* stock was grazed per day, an amount equivalent to 48% (± 17%) of the potential daily chlorophyll *a* production when inorganic nitrogen levels are enhanced and 82% (± 32%) of the potential production in the absence of additional nitrogen.

Autumn

During April to June, initial chlorophyll *a* concentrations at mid-depth were fairly constant from site to site, with an overall average of 1.4 (± 0.5) μg chl L^{-1}. Mean growth coefficients did not vary greatly, however, they were much lower than those observed in spring and summer. The overall mean growth rate for nitrogen enhanced treatments was 0.6 (± 0.3)

μ d^{-1}, which equates to an average of 0.8 (± 0.4) chlorophyll *a* doublings per day. For treatments with additional nitrogen, mean potential production was 1.3 μg chl $L^{-1}d^{-1}$.

Grazing rates were highly variable during autumn with station averages ranging from 0.01 d^{-1} (Werribee) to 0.5 d^{-1} at Sandringham. The mean grazing rate for all sites examined during autumn indicates 18% (± 18%) of initial chlorophyll *a* stock was grazed per day. This is equivalent to the grazing of 47% (± 46%) of the potential daily chlorophyll *a* production when nutrients are added and 64% (± 49%) when nutrients are limiting.

Winter

The mid-depth chlorophyll *a* concentrations were highest during winter (July to September). Over all sites, the average was 2.5 (± 1.3) μg chl L^{-1}. Mean growth rates in nutrient enhanced treatments varied with site, with the lowest mean rate for midbay waters (0.1 ± 0.2 μ d^{-1}) and the highest for Wooley's Buoy (0.6 ± 0.3 μ d^{-1}). All sites combined, the average winter growth rate was 0.3 (± 0.3) μ d^{-1} (about 0.4 chlorophyll *a* doublings per day). Estimates of potential production in nutrient enhanced treatments were highly variable; station means ranged from 0.2 μg chl $L^{-1}d^{-1}$ (midbay) to 2.3 μg chl $L^{-1}d^{-1}$ (Wooley's Buoy).

Grazing rates in winter were variable but generally low, particularly for midbay and Werribee waters (g < 0.1 d^{-1}). The mean grazing rate for all sites indicates 11 % (± 11 %) of the initial chlorophyll *a* stock was grazed per day, an amount equivalent to 33% (± 38) of the potential daily chlorophyll *a* production when inorganic nitrogen is added and 62% (± 46%) in the absence of additional nutrients.

Annual

The overall average mid-depth chlorophyll *a* concentration, representing sites sampled during October 1994 to mid-September 1995, was 1.3 μg chl L^{-1}. The mean nitrogen enhanced growth rate for the combined sites was 1.0 (± 0.7) μ d^{-1}, with average potential production estimated at 2.3 (± 2.3) μg chl $L^{-1}d^{-1}$.

For all experiments, the mean microzooplankton grazing rate indicates an average of 22% (± 17%) of the initial chlorophyll *a* standing stock was grazed per day. This is equivalent to 38% (± 31%) of the potential total chlorophyll *a* production when nitrogen is not limiting. The estimated annual grazing impact, without additional inorganic nitrogen, is 68% (± 39%) of potential chlorophyll *a* production.

Table 10. Summary of results (mean ± st dev) for the total population with estimates of grazing impact by season. Note: negative grazing coefficients, which were not significantly different from 0 (see Appendix 4) were considered as 0 values for grazing impact estimates. See Appendix 8 for individual estimates of grazing impact. Site locations are: Wooley's Buoy (Stn 2), Sandringham (Stn 3), Werribee (Stn 5), Clifton Springs (Stn 6) and midbay sites (Stn 9 & 11). +N (Nitrogen enhanced), -N (Nitrogen not added).

Season 94-95	Sites	N	Initial chl *a* stock P_o ($\mu g\ L^{-1}$)	+N Chl *a* Growth Rate k ($\mu\ d^{-1}$)	Doublings chl *a* per day k/ln2	Grazing Rate g (d^{-1})	+N Potential chl *a* production $P_p=(P_o e^{k})-P_o$ ($\mu g\ L^{-1}\ d^{-1}$)	+N Actual chl *a* production $P_a=(P_o e^{(k-g)})-P_o$ ($\mu g\ L^{-1}\ d^{-1}$)	Initial chl *a* stock grazed d^{-1} $(1-e^{-g})$ x 100 (%)	+N Potential production grazed d^{-1} $(P_p-P_a)/P_p$ x 100 (%)	-N Potential production grazed d^{-1} * (%)	N
Spring	all	11	0.6 ± 0.3	1.10 ± 0.48	1.6 ± 0.7	0.18 ± 0.14	1.5 ± 1.1	1.2 ± 0.9	16 ± 11	23 ± 17	47 ± 34	5
(Oct-Dec)	2	3	0.6 ± 0.4	1.24 ± 0.40	1.8 ± 0.6	0.32 ± 0.08	1.6 ± 1.0	1.0 ± 0.8	27 ± 6	40 ± 12	46	1
	3	2	0.8 ± 0.3	0.72 ± 0.56	1.0 ± 0.8	0.05 ± 0.08	1.1 ± 1.3	1.0 ± 1.1	5 ± 7	8 ± 11	85	1
	5,6	4	0.8 ± 0.1	1.20 ± 0.57	1.7 ± 0.8	0.15 ± 0.15	2.1 ± 1.4	1.7 ± 1.0	13 ± 13	18 ± 17	37 ± 52	2
	9,11	2	0.3 ± 0.1	1.05 ± 0.55	1.5 ± 0.8	0.15 ± 0.02	0.7 ± 0.6	0.5 ± 0.5	14 ± 2	23 ± 4	30	1
Summer	all	12	1.0 ± 0.4	1.71 ± 0.41	2.5 ± 0.6	0.51 ± 0.23	4.6 ± 2.7	2.5 ± 1.8	38 ± 14	48 ± 17	82 ± 32	10
(Jan-Mar)	2	4	0.8 ± 0.2	1.97 ± 0.52	2.8 ± 0.7	0.63 ± 0.26	4.8 ± 2.3	2.2 ± 1.2	45 ± 15	53 ± 16	92 ± 17	3
	3	2	1.5 ± 0.3	1.88 ± 0.10	2.7 ± 0.1	0.32 ± 0.16	8.4 ± 2.6	5.5 ± 0.6	27 ± 12	32 ± 13	75 ± 35	2
	5,6	3	1.2 ± 0.5	1.56 ± 0.25	2.2 ± 0.4	0.53 ± 0.28	4.4 ± 1.2	2.2 ± 1.3	40 ± 17	51 ± 24	67 ± 58	3
	9,11	3	0.6 ± 0.3	1.38 ± 0.29	2.0 ± 0.4	0.46 ± 0.18	2.1 ± 1.9	1.0 ± 0.7	36 ± 11	48 ± 12	95 ± 7	2
Autumn	all	9	1.4 ± 0.5	0.58 ± 0.30	0.8 ± 0.4	0.22 ± 0.22	1.3 ± 1.1	0.8 ± 0.8	18 ± 18	47 ± 46	64 ± 49	9
(Apr-June)	2	3	1.3 ± 0.2	0.70 ± 0.11	1.0 ± 0.2	0.17 ± 0.29	1.4 ± 0.5	1.1 ± 0.9	14 ± 23	29 ± 42	52 ± 68	3
	3	1	2.1	0.65	0.9	0.50	2.0	0.4	39	82	80	1
	5	2	1.4 ± 0.9	0.48 ± 0.22	0.7 ± 0.3	0.01 ± 0.01	1.0 ± 1.1	1.0 ± 1.0	1 ± 1	2 ± 2	0	2
	9,11	3	1.2 ± 0.4	0.51 ± 0.53	0.7 ± 0.8	0.33 ± 0.10	1.3 ± 1.8	0.6 ± 1.1	28 ± 8	82 ± 31	100 ± 0	3
Winter	all	9	2.5 ± 1.3	0.32 ± 0.27	0.4 ± 0.4	0.13 ± 0.14	1.0 ± 1.1	0.7 ± 1.0	11 ± 11	33 ± 38	62 ± 46	9
(July-Sept)	2	3	3.4 ± 1.7	0.58 ± 0.26	0.8 ± 0.4	0.13 ± 0.20	2.3 ± 0.8	1.9 ± 1.1	11 ± 17	20 ± 29	50 ± 70	3
	3	2	2.0 ± 0.1	0.25 ± 0.15	0.4 ± 0.2	0.24 ± 0.16	0.6 ± 0.4	0.0 ± 0.0	20 ± 13	92 ± 9	92 ± 11	2
	5	1	1.8	0.34	0.5	0.08	0.7	0.5	8	26	28	1
	9,11	3	2.1 ± 1.4	0.10 ± 0.17	0.1 ± 0.2	0.06 ± 0.02	0.2 ± 0.3	0.1 ± 0.2	6 ± 2	8 ± 14	0 ± 0	3
All	all	41	1.3 ± 1.0	0.99 ± 0.66	1.4 ± 1.0	0.27 ± 0.24	2.3 ± 2.3	1.4 ± 1.4	22 ± 17	38 ± 31	68 ± 39	34

* potential production grazed was estimated by applying the grazing coefficient to the chlorophyll *a* growth data for an undiluted seawater treatment which received no additional nitrogen. See Appendix 4 for estimates of growth rate without nitrogen addition.

4. DISCUSSION

4.1. The dilution method and its use in oligotrophic waters

There are a number of assumptions and limitations associated with the use of the dilution method for estimating microzooplankton grazing and phytoplankton growth rates, and these are reviewed in detail in Landry (1993). Some of the assumptions and requirements of the dilution method, however, are difficult to examine. In particular, the grazing coefficient estimated by the dilution method reflects the mean number and composition of the grazers, which are present during the incubation period. Changes in grazer density over the incubation period, however, are not easily assessed and in this study, they were not quantified. The initial grazer density (represented by dilution level), rather than the average grazer density, was used in determinations of the growth and grazing coefficients. Although the application of initial dilution levels, in representing relative concentrations of grazers, has been commonly practiced, it is likely to introduce some error in estimating growth and grazing coefficients.

Procedures of the dilution method generally include the pre-screening of experimental seawater through a 200 μm mesh to remove larger zooplankters. The pre-screening process, however, may cause considerable damage to large phytoplankton cells and to fragile nanoflagellates and ciliates. In this study, we chose not to pre-screen in order to minimise grazer mortality and because concentrations of holozooplankton in Port Phillip Bay are known to be quite low compared to other temperate bays (MMBW & FWD 1973, Axelrad *et al.* 1981). Kimmerer and McKinnon (1985) reported net zooplankton (>200 μM) mean densities of only 1480 m^{-3} or <2 ind L^{-1}. Because the grazing rates estimated in this study include the grazing impact of microzooplankters and some mesozooplankters, grazing rate estimates may be more representative of the broader zooplankton community in Port Phillip Bay. And because mesozooplankton predate on the microzooplankton (review in Pierce and Turner 1992) as well as graze on phytoplankton, they have the capacity to limit heterotrophic nanoflagellate and ciliate population growth during incubation and thus may reduce errors associated with the use of initial grazer densities (dilution levels) for estimating growth and grazing coefficients.

The dilution method assumes that chlorophyll *a* can be used as a reasonable measure of the growth of phytoplankton. It seems that this technique may be most appropriate for use in waters, which do not require the addition of nutrients to ensure exponential microalgal growth. Nutrient enhancement undoubtedly affects the physiological state and growth rates of phytoplankton cells from oligotrophic waters. In this study, samples incubated with additional inorganic nitrogen during the spring and summer, when ambient levels were low (<0.4 μM NH_4), often showed higher post-incubation levels of chlorophyll *a* per cell within cells >2 μm. Observed growth in chlorophyll *a* did not always correspond to increases in cell concentration or cell size. It seems that the nutrient history of cells, as well as temperature (Geider 1987), may have been factors contributing to enhanced cellular levels of chlorophyll *a* when nutrients were added, particularly during summer. The experiments conducted for the midbay sites, which are likely to experience less dramatic fluctuations in NH_4 concentration, and

where picophytoplankton predominate for much of the year, showed lower percent increases in chlorophyll *a* content per cell than those observed for the coastal sites. This study suggests that the introduction of additional inorganic nitrogen may at times raise cellular concentrations of chlorophyll *a* in Port Phillip Bay phytoplankton. This is an important issue as it is the chorophyll *a* data from the dilution series which is used to estimate phytoplankton growth rates. When the cellular level of chlorophyll *a* is elevated following incubation with additional nitrogen, then phytoplankton growth rates determined using the Dilution Method will be overestimated. Though the grazing rate estimates are unaffected by changes in chlorophyll *a* content per cell, it may be prudent to use an alternate method for future determinations of phytoplankton growth rates in Port Phillip Bay. Studies conducted *in situ* are also preferable to those which are conducted in the laboratory under artificial light conditions.

4.2. Interpretation of phytoplankton growth data

Seasonal patterns in total phytoplankton growth were apparent for all stations, with growth rates being highest during spring and summer and lowest during winter, for both nutrient enhanced and nonspiked treatments.

The nitrogen enhanced growth rates were often very high in summer, indicating up to three chlorophyll *a* doublings per day, which cannot be considered reflective of phytoplankton growth in the Bay at the time of sampling. Maximum growth rates observed in nitrogen spiked treatments exceeded those which have been reported for dilution experiments conducted elsewhere (Table 11). The rates observed in this study, however, provide an indication of chlorophyll *a* growth when additional dissolved inorganic nitrogen is added, and the likely growth response of phytoplankton in the event of increased nutrient loading to Port Phillip Bay. Growth rates within the treatments which did not receive additional nutrients are probably conservative estimates of phytoplankton growth for Bay waters due to potential nutrient exhaustion during incubation, especially during the summer months. *In situ* growth rates at the time of sampling were probably similar to, or slightly higher, than the nonspiked growth rates.

Table 11. A comparison of reported phytoplankton (chlorophyll *a*) growth rates (k) and microzooplankton grazing rates (g) determined using the dilution technique. Table adapted and extended from Gallegos (1989). * nitrogen added to incubation bottles.

Study Location	Range k (d^{-1})	Range g(d^{-1})	Source
Coastal Waters, Washington, USA	0.45-0.63 *	0.07-0.28	Landry & Hassett 1982
Kaneohe Bay, Hawaii, USA	1.40-2.00 *	0.10-0.40	Landry *et al.* 1984
Celtic Sea, British Isles	0.16-0.35 *	0.36-1.04	Burkill *et al.* 1987
Jones Sound, Canadian Arctic	0.06-0.34 *	0.02-0.17	Paranjape 1987
Halifax Harbour, Nova Scotia, CA	0.24-1.68	0.42-2.01	Gifford 1988
Rhode River, Maryland, USA	0.48-2.30 *	0.19-2.01	Gallegos 1989
Subarctic Pacific Ocean	0.25-0.67	0.05-0.31	Strom & Welschmeyer 1991
Norwegian fjords	0.37-0.67 *	0.43-0.56	Verity & Vernet 1992
Chesapeake Bay, USA	0.00-2.15	0.00-1.60	McManus & Ederington-Cantrell 1992
Hiroshima Bay, Seto Inland Sea	0.33-1.87	0.14-1.23	Kamiyana 1994
Four League Bay, Gulf of Mexico	0.46-2.14	0.32-2.11	Dagg 1995
Port Phillip Bay, Australia	0.00-2.65 *	0.00-0.87	This study

4.3. Phytoplankton growth responses to temperature and NH_4 addition

In spring and summer, when inorganic nitrogen levels were low, the addition of NH_4 resulted in high growth rates (k = 1-2 μ d^{-1}) for all three phytoplankton size fractions, particularly the nano and micro fractions. Without additional nitrogen, the growth rates of the nano and micro fractions during summer were limited (k generally < 0.5 μ d^{-1}). Relatively higher growth rates of the pico fraction under summer temperatures and low nitrogen conditions demonstrated that the picophytoplankton were better able to utilise available and regenerated pools of dissolved inorganic nitrogen and thus outcompete the nano and microphytoplankton. This phenomenon is commonly observed in oligotrophic waters (Fenchel 1988) and explains the predominance of the pico fraction (up to 65% of total chlorophyll *a*) in mid-depth waters at most sites during the summer months.

During the autumn and winter months, when ambient NH_4 levels were relatively high (0.4-1 μM), growth rates were reduced (k generally <0.6 μ d^{-1}) and nutrient enhanced growth rate estimates were very similar to rates determined without additional nitrogen. Because ambient nutrient levels appeared to be less limiting for microalgal growth during autumn and winter, estimates determined for this period may be good approximations of *in situ* growth rates.

Phytoplankton growth rates of the pico, nano and micro fractions were positively correlated with temperature when inorganic nitrogen was added. One would therefore expect that increased NH_4 loadings to the Bay during spring and summer would result in substantial growth, particularly of the nano- and microphytoplankton, which exhibited higher summer growth rates than the pico fraction. At temperatures >14°C, nutrient levels in bottles with undiluted seawater showed that, for a loss of 1 μM NH_4, chlorophyll *a* concentration increased by 1-2.5 μg L^{-1}. Experiments conducted during winter indicated no net decrease in NH_4 concentration and little or no increases in chlorophyll *a* in undiluted seawater treatments. Though standing stocks of chlorophyll *a* were relatively high in winter (1-5 μg L^{-1}), phytoplankton growth rates were low (mean = 0.32 μ d^{-1}). This suggests that if inorganic nitrogen loads are further increased during winter, such inputs would not be immediately utilised by phytoplankton. This relationship should be further investigated in experiments specifically designed to assess year-round inorganic nitrogen utilisation and phytoplankton growth.

4.4. Grazing rates

Seasonal trends in community grazing rate were apparent at most sites, with maximum grazing rates in summer (mean = 0.51 d^{-1}) and with low grazing impact in winter (mean = 0.13 d^{-1}). High summer grazing coefficients observed in this study have also been noted in other estuarine and coastal water systems (Gallegos 1989, Kamiyana 1994, Dagg 1995). The grazing rates estimated for Port Phillip Bay waters also fall within the range of values reported for other grazing studies which have used the dilution method (Table 11).

The high grazing rates exhibited during summer coincided with the seasonal increase in abundance of naulpii, copepodites and bivalve larvae. But, due to limited data on zooplankton composition in this study, the relative importance of the various grazers, including heterotrophic nanoflagellates and ciliates, remains unknown.

At most sites, grazing rates on the total phytoplankton population tended to mirror total chlorophyll *a* growth rates, with high grazing rates observed during peaks in chlorophyll *a* growth and reduced grazing rates when chlorophyll *a* growth rates were low. The grazing impact on the fractions also tended to follow seasonal patterns in growth rates associated with the fractions. The grazing coefficients, however, tended to be higher for the pico and nano fractions than for the microphytoplankton suggesting that small heterotrophs in Port Phillip Bay are most important in regulating phytoplankton cells <20 μm. Similar results have been reported for other studies (Gifford 1985, Verity 1985).

The grazing rate on the pico fraction was highest for the midbay sites while the coastal sites, Werribee and Wooley's Buoy, frequently showed highest grazing rates on the nano fraction. Low but significant levels of grazing on the micro fraction were shown at some stations in summer, however, 75% of experiments during this study exhibited grazing rate estimates on the micro fraction which were not significantly different

from zero ($p<0.05$). Strom and Welschmeyer (1991) also report very low grazing rates of microzooplankton on large phytoplankton cells in oligotrophic waters.

Negative grazing rates, which were commonly observed for the micro fraction during this study, were often associated with low initial chlorophyll *a* levels within the micro fraction. Potential errors associated with the size fractionation process and the frequent occurrence of gelatinous and amorphous material in incubated seawater may have contributed to low and negative grazing coefficients. Under-representation of the larger zooplankton in the incubation bottles could also be a factor. Interestingly, negative grazing coefficients for the microphytoplankton were most frequently observed for the western bay sites, Werribee and Clifton Springs. On only one occasion (June 1995) was the grazing coefficient for the micro fraction at Werribee significantly greater than zero ($p<0.05$). This is of interest as the Werribee site receives relatively large inputs of NH_4 from the Werribee Treatment Plant (Murray 1994), which could result in relatively high production of large diatoms and dinoflagellates within the micro fraction size range (>20 μm). Sedimentation of large cells is likely to occur if they are not grazed within the water column.

4.5. Total grazing impact

Estimates of grazing impact determined in this study show much temporal and spatial variability but general seasonal trends suggest that, over all sites, the mean grazing impact on the initial phytoplankton standing stock was approximately 16% in spring, 38% in summer, 18% in autumn and 11% in winter. High chlorophyll *a* standing stocks in winter reflect the reduced grazing pressure observed during this period.

Baywide estimates of grazing impact on potential production during summer averaged 48% when nitrogen was added and 82% when phytoplankton growth was not enhanced by nutrient addition. Potential production calculations, for nitrogen enhanced treatments (Table 10), are likely to be overestimates, particularly during summer when ambient nutrient levels were low. Overestimates of daily growth would result in an underestimation of grazing impact on phytoplankton production. In contrast, potential production determined from growth rates without nitrogen addition may be underestimated if incubated phytoplankton were more nutrient limited than phytoplankton *in situ*. This scenario would result in overestimation of grazing impact, which is also most likely to occur for the summer months.

Mean estimates of grazing impact on algal production during autumn, winter and spring were in the range of 23-48% (nitrogen enhanced) and 47-64% (nonspiked) of daily production. The nonspiked estimates may be reasonable estimates of *in situ* grazing impact as most estimates from studies conduct elsewhere suggest that microzooplankton graze between 40% and 60% of the primary production (Verity 1986, Gifford 1988, Strom and Welschmeyer 1991, and others).

Overall, it appears that the microzooplankton are currently very important in controlling the phytoplankton population, and perhaps even the size structure of phytoplankton, in Port Phillip Bay. Though increased microalgal growth rates,

resulting from potentially larger inputs of nutrient to the Bay, could decrease the grazing impact of current concentrations of micrograzers, it is also possible that greater phytoplankton production may result in increases in the size of the zooplankton population in Port Phillip Bay.

4.6. Other potentially important grazers

Grazing by the larvacean, *Oikopleura dioica*, was not included in this study due to apparent high larvacean mortalities prior to incubation. However, they were present during November to June and in 65% of all experiments. Larvacean densities peaked during summer with concentrations of up to 7 ind L^{-1}. Zooplankton surveys conducted in previous years showed that *Oikopleura dioica* comprised approximately 20% of holozooplankton (>200 μm) in Port Phillip Bay in 1983-84 (Kimmerer and McKinnon 1985) and in 1986 (Fancett 1988). Larvaceans are capable of efficiently clearing both pico- and nanophytoplankton (Deibel and Lee 1992) and when abundant, may clear significantly more of the water column than crustacean zooplankton (Knoechel and Steel-Flynn 1989). It is therefore highly probable that *Oikopleura dioica* has a significant impact on the pico- and nanophytoplankton production in the Bay, particularly during nonwinter months.

The grazing and predation potential of *Noctiluca scintillans,* which is a relatively new resident of Port Phillip Bay waters, may be great and needs to be monitored. These large flagellates (up to 2000 μM diam) have a tendency to congregate near the surface, are polyphagous (without any food preference) and have the capacity to efficiently clear a broad range of food particles from the water column (Uhlig and Sahlig 1990). The fact that they feed on microzooplankton as well as phytoplankton was confirmed by occasional observations of bivalve larvae and nauplii in *Noctiluca* food vacuoles. In this study, the highest mid-depth densities of *Noctiluca* were found in seawater collected from the eastern side of the Bay during December and early January (>80 ind L^{-1}). Their concentrations in surface waters were probably, at times, much greater.

5. ACKNOWLEDGMENTS

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Appendix 1. Summary of the sample collection dates, water temperature (*in situ* and incubation), initial NH_4 levels and initial chlorophyll *a* concentrations. (nd = not determined)

Station Location	Date of Sample Collection	Temp (°C)	Initial NH_4 (µM)	Initial Chlorophyll *a*			
				Total (μgL^{-1})	% Pico	% Nano	% Micro
Wooley's Buoy (Stn 2)	12-Oct-94	12.5	nd	0.34	55	41	4
	9-Nov-94	14.5	nd	1.06	27	56	17
	6-Dec-94	18.0	0.85	0.43	52	44	4
	4-Jan-95	20.5	0.46	0.62	18	22	60
	31-Jan-95	21.8	nd	0.58	38	36	26
	27-Feb-95	21.5	0.12	0.95	37	37	26
	27-Mar-95	19.0	0.11	0.85	45	25	31
	26-Apr-95	14.5	0.73	1.21	27	37	36
	23-May-95	14.0	nd	1.54	43	45	12
	20-Jun-95	11.5	0.99	1.17	48	37	16
	18-Jul-95	10.5	nd	4.03	38	32	28
	15-Aug-95	10.5	0.44	4.66	11	12	77
	11-Sep-95	10.0	0.40	1.46	45	33	22
Sandringham (Stn 3)	13-Oct-94	12.5	nd	0.56	42	53	4
	11-Dec-94	17.0	0.80	1.00	27	54	19
	24-Jan-95	21.5	0.13	1.70	32	48	19
	29-Mar-95	18.5	0.28	1.29	27	17	57
	24-May-95	14.0	nd	2.15	32	32	36
	17-Jul-95	11.0	0.81	1.91	31	33	37
	14-Sep-95	11.0	0.48	2.08	56	41	3
Werribee (Stn 5)	13-Oct-94	13.1	nd	0.87	30	54	15
	10-Nov-94	14.1	nd	0.78	41	51	8
	4-Jan-95	20.1	0.21	1.03	67	28	4
	3-Mar-95	21.5	0.08	0.83	35	39	26
	24-Apr-95	15.0	0.36	2.02	38	23	39
	22-Jun-95	11.0	0.41	0.78	38	54	8
	14-Aug-95	10.5	0.72	1.76	36	20	44
Clifton Springs (Stn 6) midbay NE (Stn 9)	14-Oct-94	13.0	nd	0.70	51	43	6
	11-Nov-94	17.0	nd	0.78	43	40	16
	28-Mar-95	19.0	nd	1.83	33	13	54
	5-Jan-95	21.5	0.08	0.40	55	37	8
	1-Mar-95	21.5	0.09	0.95	51	26	23
	22-May-95	13.0	nd	1.22	48	38	14
	20-Jul-95	10.0	nd	3.67	17	70	13
	10-Sep-95	10.0	0.47	1.20	68	30	3
midbay SW (Stn 11)	14-Oct-94	12.6	nd	0.23	44	47	9
	10-Dec-94	20.1	0.65	0.35	51	42	7
	2-Feb-95	21.5	0.24	0.49	54	32	14
	27-Apr-95	15.0	0.36	1.68	31	18	51
	21-Jun-95	12.0	0.75	0.80	47	42	11
	19-Aug-95	10.0	0.33	1.40	21	26	53

Appendix 2. Phytoplankton cell concentration and cell volume (cells >2 μm) in undiluted, unfractionated samples before and after 24 hr incubation with 1-3 μM inorganic nitrogen.

Date	Site	Cell Concentration (L^{-1})		%	Cell Volume ($\mu m^3 L^{-1}$)		%
		Initial	Final	diff	Initial	Final	diff
12-Oct-94	2*	7.6×10^{2}	8.4×10^{2}	11	4.3×10^{6}	5.9×10^{6}	37
14-Oct-94	3*	5.6×10^{2}	6.0×10^{2}	7	1.7×10^{6}	3.3×10^{6}	94
14-Oct-94	5	4.8×10^{4}	2.0×10^{4}	-59	9.0×10^{7}	1.5×10^{7}	-83
15-Oct-94	6	3.4×10^{4}	2.0×10^{4}	-39	1.7×10^{7}	1.1×10^{7}	-33
14-Oct-94	11	8.0×10^{4}	5.5×10^{4}	-32	4.0×10^{7}	7.2×10^{7}	80
9-Nov-94	2	8.7×10^{4}	1.0×10^{5}	19	7.0×10^{7}	4.6×10^{7}	-35
10-Nov-94	5	2.5×10^{5}	nd	nd	1.3×10^{8}	nd	nd
11-Nov-94	6	4.8×10^{4}	7.5×10^{4}	57	3.4×10^{7}	7.4×10^{7}	119
7-Dec-94	2*	1.3×10^{3}	1.1×10^{4}	750	5.2×10^{6}	1.9×10^{7}	265
11-Dec-94	3	1.5×10^{5}	9.6×10^{4}	-38	2.0×10^{8}	1.6×10^{8}	-21
11-Dec-94	11	3.8×10^{4}	5.7×10^{4}	49	1.0×10^{8}	1.1×10^{8}	9
4-Jan-95	2	2.2×10^{5}	nd	nd	3.1×10^{8}	nd	nd
4-Jan-95	5	1.6×10^{5}	nd	nd	4.5×10^{7}	nd	nd
6-Jan-95	9	4.3×10^{4}	nd	nd	5.8×10^{7}	nd	nd
31-Jan-95	2	3.0×10^{5}	3.8×10^{5}	29	3.6×10^{9}	3.8×10^{9}	4
24-Jan-95	3	9.0×10^{5}	9.0×10^{5}	0	7.2×10^{9}	6.3×10^{9}	-12
2-Feb-95	11	9.5×10^{4}	1.9×10^{5}	102	1.1×10^{9}	1.2×10^{9}	5
27-Feb-95	2	3.9×10^{4}	1.1×10^{5}	174	2.6×10^{9}	3.2×10^{9}	34
4-Mar-95	5	1.3×10^{5}	1.3×10^{5}	-1	1.5×10^{9}	1.1×10^{9}	-31
1-Mar-95	9	1.4×10^{5}	1.7×10^{5}	25	4.1×10^{9}	6.2×10^{9}	52
27-Mar-95	2	1.8×10^{5}	2.5×10^{5}	35	6.8×10^{9}	1.4×10^{10}	112
29-Mar-95	3	1.2×10^{6}	8.8×10^{5}	-29	1.7×10^{10}	1.5×10^{10}	-12
28-Mar-95	6	4.7×10^{5}	5.3×10^{5}	12	5.2×10^{10}	6.0×10^{10}	15
26-Apr-95	2	7.3×10^{5}	9.4×10^{5}	29	3.2×10^{9}	3.9×10^{9}	22
24-Apr-95	5	6.6×10^{5}	1.0×10^{6}	53	8.3×10^{8}	4.2×10^{9}	405
27-Apr-95	11	1.2×10^{6}	1.4×10^{6}	12	7.1×10^{9}	2.9×10^{9}	-58
23-May-95	2	7.2×10^{5}	nd	nd	5.1×10^{9}	nd	nd
24-May-95	3	1.3×10^{6}	1.4×10^{6}	8	3.4×10^{9}	3.2×10^{9}	-4
22-May-95	9	3.5×10^{5}	4.6×10^{5}	31	8.8×10^{8}	1.1×10^{9}	20
20-Jun-95	2	2.7×10^{5}	5.6×10^{5}	104	6.4×10^{8}	1.7×10^{9}	163
22-Jun-95	5	1.7×10^{5}	2.8×10^{5}	65	7.9×10^{8}	1.2×10^{9}	53
21-Jun-95	11	1.6×10^{5}	2.2×10^{5}	37	2.5×10^{8}	5.6×10^{8}	120
18-Jul-95	2	1.3×10^{6}	1.8×10^{6}	42	9.5×10^{9}	1.1×10^{10}	16
17-Jul-95	3	4.6×10^{5}	8.0×10^{5}	71	3.1×10^{9}	3.6×10^{9}	17
20-Jul-95	9	5.6×10^{5}	7.6×10^{5}	34	2.6×10^{9}	2.7×10^{9}	2
15-Aug-95	2	5.3×10^{6}	9.5×10^{5}	-82	2.3×10^{10}	1.1×10^{10}	-52
14-Aug-95	5	3.6×10^{5}	3.4×10^{6}	836	1.2×10^{9}	1.5×10^{10}	1180
19-Aug-95	11	1.4×10^{6}	1.3×10^{6}	-7	5.9×10^{9}	5.5×10^{9}	-7
11-Sep-95	2	2.4×10^{5}	3.2×10^{5}	36	3.9×10^{8}	4.7×10^{8}	22
14-Sep-95	3	1.3×10^{6}	1.4×10^{6}	8	4.1×10^{9}	3.9×10^{9}	-4
10-Sep-95	9	1.7×10^{5}	1.9×10^{5}	12	2.2×10^{8}	2.4×10^{8}	7

nd = not determined * cell counts very low (probably due to incomplete fixation)

Appendix 3. Dominant phytoplankton species (<2 μm) and cell concentrations in undiluted experimental bottles before and after 24 hr incubation with 1-3 μM inorganic nitrogen.

Date of Incubation	Site	Initial Dominant Species	Initial cells L^{-1}	Final Dominant Species	Final cells L^{-1}
14-Oct-94	5	*Thalassiosira mala*	4.7×10^4	*Thalassiosira mala*	1.8×10^4
15-Oct-94	6	*Pyramimonas sp.*	1.5×10^4	*Pyramimonas sp.*	1.0×10^4
14-Oct-94	11	*Pseudonitzschia pungens*	1.4×10^4	*Teleaulax amphioxeia*	1.4×10^4
		Leucocryptos marina	3.8×10^4	*Proboscia alata*	2.8×10^4
9-Nov-94	2	*Thalassiosira mala*	4.9×10^4	*Thalassiosira mala*	3.6×10^4
10-Nov-94	5	*Thalassiosira mala*	2.2×10^5	*Thalassiosira mala*	5.2×10^4
11-Nov-94	6	*Plagioselmis prolonga*	2.5×10^4	*Katodinium rotundatum*	3.9×10^4
11-Dec-94	3	*Pseudonitzchia pungens*	6.2×10^4	*Pseudonitzchia pungens*	1.6×10^4
				Imantonia rotunda	1.6×10^4
11-Dec-94	11	*Leucocryptos marina*	1.7×10^4	*Leucocryptos marina*	2.1×10^4
				Cymbomonas tetramitiformis	1.3×10^4
				Pseudonitzchia pungens	1.2×10^4
4-Jan-95	2	*Corymbellus aureus*	1.3×10^5	nd	
4-Jan-95	5	*Leucocryptos marina*	4.3×10^4	nd	
		Hemiselmis virescens	6.1×10^4		
6-Jan-95	9	*Plagioselmis prolonga*	1.2×10^4	nd	
31-Jan-95	2	*Rhizosolenia delicatula*	1.4×10^5	*Rhizosolenia delicatula*	1.2×10^5
		Guinardia flaccida	5.8×10^4	*Guinardia flaccida*	6.5×10^4
24-Jan-95	3	*Skeletonema costatum*	2.6×10^5	*Skeletonema costatum*	2.0×10^5
		Plagioselmis prolonga	1.2×10^5	*Plagioselmis prolonga*	6.7×10^4
		Leucocryptos marina	6.7×10^4	*Leucocryptos marina*	8.0×10^4
2-Feb-95	11	*Leucocryptos marina*	3.2×10^4	*Pseudonitzchia pungens*	5.3×10^4
				Rhizosolenia delicatula	3.4×10^4
27-Feb-95	2	*Proboscia alata*	1.9×10^4	*Proboscia alata*	2.0×10^4
				Plagioselmis prolonga	6.7×10^4
4-Mar-95	5	*Plagioselmis prolonga*	2.2×10^4	*Plagioselmis prolonga*	2.1×10^4
				Leucocryptos marina	2.1×10^4
1-Mar-95	9	*Proboscia alata*	1.9×10^4	*Proboscia alata*	2.5×10^4
		Pyramimonas spp.	2.4×10^4	*Pyramimonas spp.*	2.5×10^4
27-Mar-95	2	*Proboscia alata*	6.2×10^4	*Proboscia alata*	7.2×10^4
		Leucocryptos marina	2.2×10^4	*Leucocryptos marina*	2.4×10^4
				Gymnodinium sp.	3.6×10^4
29-Mar-95	3	*Proboscia alata*	7.2×10^4	*Proboscia alata*	1.0×10^5
		Skeletonema costatum	6.3×10^5	*Skeletonema costatum*	1.7×10^5
		Chaetoceros sp.	1.6×10^5	*Chaetoceros sp.*	1.2×10^5
				Cymbomonas tetramitiformis	1.2×10^5
28-Mar-95	6	*Proboscia alata*	2.5×10^5	*Proboscia alata*	3.4×10^5
26-Apr-95	2	*Pseudonitzchia pungens*	9.1×10^4	*Pseudonitzchia pungens*	1.6×10^5
		Phaeocystis pouchettii	1.2×10^5	*Phaeocystis pouchettii*	1.1×10^5
24-Apr-95	5	*Chaetoceros socialis*	5.5×10^5	*Chaetoceros socialis*	2.1×10^5
		Plagioselmis prolonga	3.1×10^4	*Plagioselmis prolonga*	1.7×10^5

nd = not determined. Data from 3 samples with very low cell counts ($<10^4$ cells L^{-1}) not included.

Appendix 3 continued.

Date of Incubation	Site	Initial Dominant Species	Initial cells L^{-1}	Final Dominant Species	Final cells L^{-1}
27-Apr-95	11	*Rhizosolenia delicatula*	5.7×10^5	*Rhizosolenia delicatula*	1.5×10^5
		Skeletonema costatum	1.4×10^5	*Skeletonema costatum*	3.1×10^5
		Phaeocystis pouchettii	1.0×10^5	*Pseudonitzchia pungens*	2.0×10^5
23-May-95	2	*Rhizosolenia delicatula*	1.5×10^5	*Rhizosolenia delicatula*	3.8×10^5
		Gymnodinium spp.	1.1×10^5		
24-May-95	3	*Rhizosolenia delicatula*	3.4×10^5	*Rhizosolenia delicatula*	3.1×10^5
		Pseudonitzchia pungens	2.3×10^5	*Pseudonitzchia pungens*	1.8×10^5
		Skeletonema costatum	1.2×10^5	*Skeletonema costatum*	1.5×10^5
22-May-95	9	*Rhizosolenia delicatula*	1.5×10^5	*Rhizosolenia delicatula*	1.7×10^5
		Pseudonitzchia pungens	6.2×10^4	*Pseudonitzchia pungens*	6.0×10^4
20-June-95	2	*Skeletonema costatum*	4.0×10^4	*Skeletonema costatum*	5.6×10^4
		Thalassiosira sp.	5.8×10^4	*Rhizosolenia delicatula*	7.3×10^4
				Plagioselmis prolonga	8.8×10^4
22-June-95	5	*Gymnodinium sp.*	2.7×10^4	*Gymnodinium sp.*	1.8×10^5
21-June-95	11	*Nitzschia closterium*	5.8×10^4	*Pseudonitzchia pungens*	3.6×10^4
		Gymnodinium sp.	1.3×10^4	*Gymnodinium sp.*	3.1×10^4
		Plagioselmis prolonga	4.3×10^4	*Plagioselmis prolonga*	4.8×10^4
18-July-95	2	*Pseudonitzchia pungens*	1.2×10^5	*Pseudonitzchia pungens*	3.0×10^5
		Gymnodinium sp.	7.4×10^5	*Gymnodinium sp.*	3.2×10^5
		Rhizosolenia setigera	9.4×10^4	*Rhizosolenia setigera*	1.6×10^5
17-July-95	3	*Pseudonitzchia pungens*	1.2×10^5	*Pseudonitzchia pungens*	1.3×10^5
				Cymbomonas tetramitiformis	7.5×10^4
20-July-95	9	*Gymnodinium sp.*	8.1×10^4	*Gymnodinium sp.*	1.2×10^5
		Pyramimonas sp.	7.3×10^4	*Pyramimonas sp.*	1.1×10^5
		Teleaulax acuta	7.6×10^4	*Teleaulax acuta*	1.1×10^5
				Thalassionema sp.	1.3×10^5
15-Aug-95	2	*Pseudonitzchia pungens*	3.9×10^6	*Pseudonitzchia pungens*	2.6×10^5
		Rhizosolenia setigera	2.1×10^5	*Rhizosolenia setigera*	1.4×10^5
		Thalassionema sp.	1.4×10^5	*Thalassionema sp.*	1.7×10^5
14-Aug-95	5	*Pseudonitzchia pungens*	1.4×10^5	*Pseudonitzchia pungens*	2.3×10^5
				Rhizosolenia setigera	3.2×10^5
19-Aug-95	11	*Rhizosolenia setigera*	2.2×10^5	*Rhizosolenia setigera*	2.5×10^5
		Pseudonitzchia pungens	3.8×10^5	*Pseudonitzchia pungens*	2.8×10^5
		P. pseudodelicatissima	2.5×10^5	*Crysochromulina sp.*	1.3×10^5
11-Sept-95	2	*Pseudonitzchia pungens*	3.6×10^4	*Pseudonitzchia pungens*	4.1×10^4
		Katodinium glaucum	3.9×10^4	*Katodinium glaucum*	5.0×10^4
14-Sept-95	3	*Leptocylindrus danicus*	9.5×10^4	*Leptocylindrus danicus*	1.0×10^5
		Pseudonitzchia pungens	5.5×10^5	*Pseudonitzchia pungens*	5.7×10^5
		Chrysochromulina sp.	1.4×10^5	*Chrysochromulina sp.*	1.4×10^5
10-Sept-95	9	*Pseudonitzchia pungens*	5.7×10^4	*Pseudonitzchia pungens*	6.4×10^4
		Leucocryptos marina	3.9×10^4	*Leucocryptos marina*	3.9×10^4

Appendix 4. Total phytoplankton population grazing and chl *a* growth coefficients determined using the Dilution Method with and without nitrogen addition.

Date of Incubation	Site	Grazing rate (g)		Growth rate (k) NH_4 added		Growth rate NH_4 not added
		g (day^{-1})	St Error	k (day^{-1})	St Error	k (day^{-1})
12-Oct-94	2	0.358	0.134	0.862	0.092	nd
14-Oct-94	3	-0.001^	0.110	0.324	0.075	nd
14-Oct-94	5	0.283^	0.125	1.210	0.086	nd
15-Oct-94	6	-0.016^	0.047	0.405	0.032	nd
14-Oct-94	11	0.133^	0.107	0.661	0.073	nd
9-Nov-94	2	0.228	0.056	1.211	0.037	nd
10-Nov-94	5	0.277	0.046	1.740	0.028	0.402
11-Nov-94	6	0.051^	0.080	1.438	0.053	-0.040
7-Dec-94	2	0.380	0.049	1.654	0.033	1.181
11-Dec-94	3	0.108	0.030	1.117	0.020	0.128
11-Dec-94	11	0.168	0.048	1.437	0.032	0.707
4-Jan-95	2	0.645	0.045	2.651	0.029	1.242
4-Jan-95	5	0.811	0.048	1.417	0.031	0.421
6-Jan-95	9	0.426	0.025	1.156	0.017	nd
31-Jan-95	2	0.868	0.086	2.095	0.047*	0.576
24-Jan-95	3	0.437	0.088	1.947	0.058	0.328
2-Feb-95	11	0.291	0.047	1.272	0.031	0.327
27-Feb-95	2	0.266	0.034	1.498	0.023	0.087
4-Mar-95	5	0.245	0.058	1.853	0.038	0.225
1-Mar-95	9	0.655	0.044	1.702	0.029	0.354
27-Mar-95	2	0.721	0.094	1.655	0.062	0.688
29-Mar-95	3	0.206	0.031	1.804	0.021	0.466
28-Mar-95	6	0.539	0.151	1.412	0.021	-0.185
26-Apr-95	2	-0.012^	0.065	0.640	0.089	-0.023
24-Apr-95	5	0.016^	0.032	0.638	0.015	-0.168
27-Apr-95	11	0.369	0.120	1.107	0.096	0.350
23-May-95	2	0.009^	0.056	0.829	0.038	0.317
24-May-95	3	0.500	0.050	0.653	0.122	0.677
22-May-95	9	0.214	0.083	0.110	0.055	-0.089
20-Jun-95	2	0.508	0.073	0.622	0.128	0.434
22-Jun-95	5	-0.210~	0.069	0.323	0.062	0.039
21-Jun-95	11	0.407	0.060	0.314	0.124	0.233
18-Jul-95	2	-0.009^	0.092	0.349	0.061	0.079
17-Jul-95	3	0.120^	0.097	0.141	0.070	0.144
20-Jul-95	9	0.076^	0.138	-0.128	0.091	-0.310
15-Aug-95	2	0.029^	0.077	0.531	0.056	-0.519
14-Aug-95	5	0.078^	0.067	0.342	0.044	0.317
19-Aug-95	11	0.064^	0.104	0.288	0.071	-0.073
11-Sep-95	2	0.366	0.077	0.867	0.099	0.369
14-Sep-95	3	0.351	0.047	0.359	0.088	0.229
10-Sep-95	9	0.041^	0.051	-0.065	0.023	-0.310

nd not determined, * standard error for chl *a* growth rate without NH_4 addition,

^ not significantly different from zero, ~ significantly less than zero.

Appendix 5. Picophytoplankton fraction (0.2-2 μm) grazing and chl *a* growth coefficients determined using the Dilution Method with and without nitrogen addition.

Date of Incubation	Site	Grazing rate (g)		Growth rate (k) NH_4 added		Growth rate NH_4 not added
		g (day^{-1})	St Error	k (day^{-1})	St Error	k (day^{-1})
12-Oct-94	2	0.431^	0.096	0.910	0.157	nd
14-Oct-94	3	-0.218^	0.218	0.320	0.198	nd
14-Oct-94	5	-0.282^	0.229	1.160	0.171	nd
15-Oct-94	6	0.361	0.290	0.552	0.066	nd
14-Oct-94	11	0.151^	0.270	0.712	0.149	nd
9-Nov-94	2	0.213^	0.053	1.596	0.116	nd
10-Nov-94	5	0.235	0.085	1.786	0.033	0.678
11-Nov-94	6	0.416	0.174	1.393	0.057	0.273
7-Dec-94	2	0.189^	0.152	1.709	0.078	1.283
11-Dec-94	3	0.273^	0.118	0.570	0.148	0.019
11-Dec-94	11	0.156^	0.224	1.541	0.101	0.808
4-Jan-95	2	0.736	0.063	1.837	0.047	0.783
4-Jan-95	5	1.147	0.052	1.484	0.034	0.793
6-Jan-95	9	0.497	0.072	1.403	0.043	nd
31-Jan-95	2	0.672	0.250	1.747	0.166*	0.684
24-Jan-95	3	0.434	0.200	1.705	0.119	0.638
2-Feb-95	11	0.424	0.179	1.450	0.132	0.558
27-Feb-95	2	0.162^	0.136	1.419	0.090	0.259
4-Mar-95	5	0.759	0.122	1.813	0.081	0.458
1-Mar-95	9	0.672	0.074	1.837	0.049	0.567
27-Mar-95	2	0.402	0.059	1.331	0.039	0.110
29-Mar-95	3	0.300^	0.269	1.674	0.120	0.756
28-Mar-95	6	0.216^	0.181	1.532	0.184	0.179
26-Apr-95	2	-0.078^	0.152	0.470	0.174	0.455
24-Apr-95	5	0.320	0.053	0.359	0.026	0.103
27-Apr-95	11	0.150^	0.261	0.106	0.208	0.075
23-May-95	2	0.126^	0.224	0.850	0.042	0.252
24-May-95	3	0.702	0.225	0.378	0.149	0.694
22-May-95	9	-0.044^	0.164	-0.674	0.109	-0.574
20-Jun-95	2	0.404	0.117	0.479	0.049	0.360
22-Jun-95	5	0.339	0.098	0.749	0.154	0.421
21-Jun-95	11	0.511	0.095	0.321	0.160	0.425
18-Jul-95	2	0.381	0.130	-0.023	0.086	-0.130
17-Jul-95	3	0.156	0.063	0.113	0.042	0.164
20-Jul-95	9	0.578	0.123	0.150	0.084	0.522
15-Aug-95	2	0.284	0.150	0.261	0.100	0.220
14-Aug-95	5	-0.057^	0.108	0.217	0.072	0.239
19-Aug-95	11	0.105	0.136	0.259	0.090	0.255
11-Sep-95	2	0.434	0.049	0.795	0.033	0.343
14-Sep-95	3	0.074^	0.043	0.182	0.028	0.190
10-Sep-95	9	0.037	0.053	-0.241	0.035	-0.072

nd not determined, * standard error for chl *a* growth rate without NH_4 addition,

^ not significantly different from zero.

Appendix 6. Nanophytoplankton fraction (2-20 μm) grazing and chl *a* growth coefficients determined using the Dilution Method with and without nitrogen addition.

Date of Incubation	Site	Grazing rate (g)		Growth rate (k) NH_4 added		Growth rate NH_4 not added
		g (day^{-1})	St Error	k (day^{-1})	St Error	k (day^{-1})
12-Oct-94	2	0.294	0.110	0.880	0.075	nd
14-Oct-94	3	0.542^	0.222	0.463	0.152	nd
14-Oct-94	5	0.342^	0.184	1.415	0.126	nd
15-Oct-94	6	-0.507~	0.168	0.211	0.115	nd
14-Oct-94	11	0.055^	0.208	0.722	0.142	nd
9-Nov-94	2	0.192^	0.185	0.811	0.123	nd
10-Nov-94	5	0.365	0.049	1.899	0.030	0.208
11-Nov-94	6	-0.044^	0.079	1.624	0.052	-0.044
7-Dec-94	2	0.384^	0.264	1.327	0.175	0.834
11-Dec-94	3	0.171^	0.087	1.300	0.058	0.296
11-Dec-94	11	0.432^	0.257	1.276	0.171	0.839
4-Jan-95	2	1.309	0.299	2.030	0.194	1.965
4-Jan-95	5	0.447	0.135	1.436	0.088	0.162
6-Jan-95	9	0.139^	0.232	0.571	0.088	nd
31-Jan-95	2	0.382	0.140	1.007	0.093*	-0.132
24-Jan-95	3	0.392	0.168	1.407	0.111	0.665
2-Feb-95	11	0.216^	0.390	0.825	0.259	0.107
27-Feb-95	2	0.665	0.161	1.680	0.107	0.231
4-Mar-95	5	0.430	0.085	1.610	0.056	0.580
1-Mar-95	9	0.438	0.068	1.332	0.045	0.260
27-Mar-95	2	0.057^	0.113	1.171	0.075	0.383
29-Mar-95	3	0.157^	0.147	2.170	0.097	0.831
28-Mar-95	6	0.292	0.070	1.238	0.042	0.520
26-Apr-95	2	0.939	0.072	1.105	0.048	0.562
24-Apr-95	5	0.078^	0.096	0.521	0.063	-0.034
27-Apr-95	11	0.284^	0.158	0.506	0.126	0.005
23-May-95	2	0.040^	0.058	0.475	0.032	0.315
24-May-95	3	0.589	0.109	0.942	0.073	1.005
22-May-95	9	0.150^	0.091	0.109	0.060	0.117
20-Jun-95	2	0.102^	0.134	0.370	0.089	0.130
22-Jun-95	5	-0.238^	0.128	0.405	0.091	0.269
21-Jun-95	11	0.197	0.065	0.090	0.089	-0.118
18-Jul-95	2	0.326	0.074	0.533	0.049	0.209
17-Jul-95	3	0.181^	0.111	0.181	0.074	0.089
20-Jul-95	9	-0.120^	0.107	-0.434	0.073	-0.648
15-Aug-95	2	0.909	0.166	1.469	0.110	1.274
14-Aug-95	5	0.893	0.160	0.883	0.106	0.933
19-Aug-95	11	0.061^	0.107	0.049	0.071	-0.304
11-Sep-95	2	0.183^	0.089	0.665	0.059	0.169
14-Sep-95	3	0.007^	0.124	-0.079	0.082	-0.092
10-Sep-95	9	-0.004^	0.074	0.002	0.049	-0.038

nd not determined, * standard error for chl *a* growth rate without NH_4 addition,

^ not significantly different from zero, ~ significantly less than zero.

Appendix 7. Microphytoplankton fraction (<20 μm) grazing and chl *a* growth coefficients determined using the Dilution Method with and without nitrogen addition.

Date of Incubation	Site	Grazing rate (g)		Growth rate (k) NH_4 added		Growth rate NH_4 not added
		g (day^{-1})	St Error	k (day^{-1})	St Error	k (day^{-1})
12-Oct-94	2	-0.325~	0.130	0.638	0.089	nd
14-Oct-94	3	-0.351~	0.112	-0.079	0.076	nd
14-Oct-94	5	-0.978~	0.127	0.377	0.089	nd
15-Oct-94	6	-0.482~	0.116	0.250	0.080	nd
14-Oct-94	11	-0.417~	0.130	-0.516	0.099	nd
9-Nov-94	2	0.128^	0.219	0.879	0.145	nd
10-Nov-94	5	-0.451^	0.227	1.739	0.140	0.462
11-Nov-94	6	-0.215^	0.252	1.287	0.167	0.447
7-Dec-94	2	-0.026^	0.092	2.000	0.061	1.052
11-Dec-94	3	-0.343^	0.153	1.191	0.102	-0.090
11-Dec-94	11	0.058^	0.147	1.714	0.089	0.496
4-Jan-95	2	0.578	0.063	2.980	0.041	1.279
4-Jan-95	5	-0.384~	0.132	0.976	0.086	0.102
6-Jan-95	9	0.297	0.117	0.778	0.080	nd
31-Jan-95	2	-0.003^	0.129	1.808	0.086*	0.186
24-Jan-95	3	0.515	0.184	2.850	0.122	0.614
2-Feb-95	11	0.100^	0.174	1.230	0.116	-0.094
27-Feb-95	2	0.172^	0.150	1.372	0.100	0.080
4-Mar-95	5	-0.194~	0.061	2.196	0.040	0.177
1-Mar-95	9	0.424	0.181	1.305	0.120	0.230
27-Mar-95	2	0.609	0.085	1.402	0.057	0.598
29-Mar-95	3	0.677	0.112	1.660	0.085	0.600
28-Mar-95	6	0.694	0.105	1.326	0.072	-1.418
26-Apr-95	2	-0.050^	0.096	1.052	0.064	-0.089
24-Apr-95	5	0.091^	0.128	1.106	0.094	-0.115
27-Apr-95	11	0.312	0.086	1.233	0.068	0.274
23-May-95	2	0.033^	0.121	1.075	0.080	0.440
24-May-95	3	0.099^	0.121	0.788	0.080	0.534
22-May-95	9	0.315^	0.161	0.448	0.107	0.057
20-Jun-95	2	0.072^	0.119	1.099	0.079	0.441
22-Jun-95	5	0.358	0.083	0.889	0.059	0.675
21-Jun-95	11	0.026^	0.125	0.133	0.111	-0.008
18-Jul-95	2	0.049^	0.142	0.599	0.094	0.201
17-Jul-95	3	0.150	0.071	0.345	0.111	0.443
20-Jul-95	9	0.135^	0.078	0.286	0.054	0.181
15-Aug-95	2	-0.204^	0.099	0.484	0.069	-1.046
14-Aug-95	5	-0.230^	0.118	0.161	0.078	0.301
19-Aug-95	11	-0.180~	0.078	0.380	0.052	-0.090
11-Sep-95	2	0.325	0.100	1.233	0.066	0.155
14-Sep-95	3	-0.220^	0.275	0.287	0.183	0.201
10-Sep-95	9	0.461^	0.100	0.559	0.147	0.398

nd not determined, * standard error for chl *a* growth rate without NH_4 addition,

^ not significantly different from zero, ~ significantly less than zero.

Appendix 8. Chlorophyll *a* production in bottles incubated with 1-3 μM inorganic nitrogen and estimates of initial stock and potential production grazed for each experiment.

Date	Site	Initial chl *a* stock P_o μg L^{-1}	Doublings chl *a* per day k/ln2	Potential chl *a* production $P_p=(P_oe^k)-P_o$ μg $L^{-1}d^{-1}$	Actual chl *a* production $P_a=(P_oe^{(k-g)})-P_o$ μg $L^{-1}d^{-1}$	Initial chl *a* stock grazed $1-e^{-g}$ μg $L^{-1}d^{-1}$ (%)	Potential production grazed $P_p- P_a$ μg $L^{-1}d^{-1}$ (%)
12-Oct-94	2	0.34	1.2	0.47	0.22	0.10 (30)	0.24 (52)
14-Oct-94	3	0.56	0.5	0.21	0.22	0.00 (0)	0.00 (0)
14-Oct-94	5	0.87	1.7	2.05	1.33	0.21 (25)	0.72 (35)
15-Oct-94	6	0.70	0.6	0.35	0.37	0.00 (0)	-0.02 (-0)
14-Oct-94	11	0.23	1.0	0.22	0.16	0.03 (12)	0.06 (26)
9-Nov-94	2	1.06	1.7	2.50	1.77	0.22 (20)	0.73 (29)
10-Nov-94	5	0.78	2.5	3.66	2.59	0.19 (24)	1.08 (29)
11-Nov-94	6	0.78	2.1	2.51	2.34	0.04 (5)	0.16 (7)
7-Dec-94	2	0.43	2.4	1.82	1.11	0.14 (32)	0.71 (39)
11-Dec-94	3	1.00	1.6	2.06	1.74	0.10 (10)	0.31 (15)
11-Dec-94	11	0.35	2.1	1.12	0.90	0.05 (15)	0.23 (20)
4-Jan-95	2	0.62	3.8	8.16	3.99	0.29 (48)	4.18 (51)
4-Jan-95	5	1.03	2.0	3.22	0.86	0.57 (56)	2.36 (73)
6-Jan-95	9	0.40	1.7	0.87	0.43	0.14 (35)	0.44 (51)
31-Jan-95	2	0.58	3.0	4.13	1.40	0.34 (58)	2.73 (66)
24-Jan-95	3	1.70	2.8	10.21	6.00	0.60 (35)	4.22 (41)
2-Feb-95	11	0.49	1.8	1.26	0.82	0.12 (25)	0.44 (35)
27-Feb-95	2	0.95	2.2	3.30	2.31	0.22 (23)	0.99 (30)
4-Mar-95	5	0.83	2.7	4.46	3.31	0.18 (22)	1.15 (26)
1-Mar-95	9	0.95	2.5	4.26	1.76	0.46 (48)	2.50 (59)
27-Mar-95	2	0.85	2.4	3.60	1.31	0.44 (51)	2.29 (64)
29-Mar-95	3	1.29	2.6	6.55	5.09	0.24 (19)	1.46 (22)
28-Mar-95	6	1.83	2.0	5.68	2.55	0.76 (42)	3.13 (55)
26-Apr-95	2	1.22	0.9	1.09	1.12	0.00 (0)	0.03 (0)
24-Apr-95	5	2.02	0.9	1.80	1.74	0.03 (2)	0.06 (3)
27-Apr-95	11	1.68	1.6	3.40	1.83	0.52 (31)	1.57 (46)
23-May-95	2	1.54	1.2	1.99	1.96	0.01 (1)	0.03 (2)
24-May-95	3	2.15	0.9	1.98	0.36	0.85 (39)	1.63 (82)
22-May-95	9	1.22	0.2	0.14	-0.12	0.24 (19)	0.26 (185)
20-Jun-95	2	1.17	0.9	1.01	0.14	0.46 (40)	0.87 (86)
22-Jun-95	5	0.78	0.5	0.30	0.55	0.00 (0)	-0.25 (-85)
21-Jun-95	11	0.80	0.5	0.29	-0.07	0.27 (33)	0.37 (124)
18-Jul-95	2	4.04	0.5	1.69	1.74	0.00 (0)	-0.05 (-3)
17-Jul-95	3	1.91	0.2	0.29	0.04	0.22 (11)	0.25 (86)
20-Jul-95	9	3.67	-0.2	-0.44	-0.68	0.27 (7)	0.24 (-54)
15-Aug-95	2	4.66	0.8	3.26	3.04	0.13 (3)	0.23 (7)
14-Aug-95	5	1.76	0.5	0.72	0.53	0.13 (8)	0.19 (26)
19-Aug-95	11	1.40	0.4	0.47	0.35	0.09 (6)	0.12 (25)
11-Sep-95	2	1.46	1.3	2.01	0.95	0.45 (31)	1.06 (53)
14-Sep-95	3	2.08	0.5	0.90	0.02	0.61 (30)	0.88 (98)
10-Sep-95	9	1.20	-0.1	-0.08	-0.12	0.05 (4)	0.05 (-60)