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Microzooplankton grazing of phytoplankton in Manukau Harbour, New Zealand

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Abstract Grazing by microzooplankton on phytoplankton in Manukau Harbour was measured by size-fractionated dilution experiments at monthly intervals from October 1994 to October 1995. Grazing rates were always highest on the < 5 μm size fraction, the smallest size fraction measured. These rates ranged from 0.3 to 1.3 d^{-1} and were highest in November and March; values did not appear to vary with grazer abundance. Grazing rates on the < 5 μm phytoplankton exceeded growth rate by more than a factor of two in June when growth rate was seasonally depressed; but for most of the year the grazing rate averaged about 90% of growth rate. Grazing rates on the 5–22 μm phytoplankton were lower in magnitude, and were a lower percentage of phytoplankton growth rate than those on the < 5 μm size fraction. Grazing on the > 22 μm phytoplankton was measurable only during the February bloom of the large diatom *Odontella sinensis*. The grazing rate was low, being a small percentage of phytoplankton growth rate in that size fraction. Mathematical simulation of the growth of the < 5 μm phytoplankton in the harbour showed that the observed grazing rates were sufficient to prevent this size fraction from blooming.

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INTRODUCTION

Since its introduction by Azam et al. (1983), the concept of the "microbial loop" has gained widespread acceptance as a more accurate description of the marine planktonic food web, compared with earlier descriptions based on linear transfer of carbon from diatoms to copepods to fish (Steele 1974). Key elements of the microbial loop hypothesis are that bacteria take up dissolved organic matter and are consumed by small heterotrophic flagellates and ciliates, which in turn are consumed by larger predators until sizes are reached that are available to crustacean zooplankton. Similarly, primary production by phytoplankton occurs over a size range that completely overlaps that of bacteria and their primary and secondary consumers. Excretion of photosynthate by phytoplankton and inefficient feeding by consumers releases dissolved organic matter, thereby completing the "loop" to bacteria. The organisms responsible for predation in the microbial loop are collectively referred to as microzooplankton, which are operationally defined as heterotrophic organisms that pass through a 200 μm screen. Taxonomically they consist of a wide range of protists (e.g., flagellates, ciliates, and heterotrophic dinoflagellates) and metazoa (e.g., rotifers and larval meroplankton). Owing to their small size, they typically have specific growth rates about as high as their bacterial and phytoplanktonic prey (Banse 1982). The potentially high grazing rates and efficient recycling of nutrients that occur in the microbial loop mean that substantial primary production by small size classes of phytoplankton can occur without net accumulation of biomass.

Blooms of phytoplankton are a common feature in nutrient-enriched aquatic systems. In Manukau Harbour (North Island) late-summer blooms of the

large diatom, *Odontella sinensis*, appear to be a recurrent feature in the north-east region, which receives discharge from a municipal wastewater treatment plant. The factor controlling phytoplankton growth rate there is believed to be underwater light, levels of which are low because of high concentrations of inorganic suspended solids (Vant 1991). Studies of size-fractionated photosynthesis and chlorophyll *a*, however, have shown that the < 5 μm, the 5–22 μm, and the > 22 μm size fractions all have similar photosynthetic responses to light (Vant & Safi 1996); but the < 22 μm size fractions do not comprise a very large proportion of the bloom. Because of its large size (> 100 μm: Vant & Budd 1993), *O. sinensis* is unlikely to be grazed by microzooplankton. The objective of the present work was to test the hypothesis that microzooplankton grazing prevents bloom formation by small phytoplankton taxa in Manukau Harbour during summer.

MATERIALS AND METHODS

Study site

Experiments to measure phytoplankton growth and microzooplankton grazing rates were carried out in Manukau Harbour monthly from October 1994 to October 1995. Manukau Harbour (37°S, 174°E) is a turbid, shallow, macrotidal estuary west of Auckland on the North Island of New Zealand. Samples for this study were collected from the region previously designated North-east (Vant & Budd 1993). Secchi depth at the site varies from < 0.5 to c. 1.4 m. Salinity ranges from 28 to 33 (practical salinity scale), with the water column generally vertically well-mixed by strong tidal currents. Mean tidal range varies from 2 m neap to 3.4 m spring. High nutrient concentrations result from a wastewater treatment plant discharge about 4 km from the sampling site. Concentrations of total inorganic nitrogen (N) generally exceed 35μ*M* but can briefly drop to < 5μ*M* when phytoplankton biomass is high in late summer owing to an annually recurring bloom of the large diatom *O. sinensis*. Soluble reactive phosphorus (P) varies from about 3 to 6μ*M* (Vant & Budd 1993). Chlorophyll *a* concentrations generally vary seasonally from 5 to 15 mg m^{-3}, except during the summer blooms of *O. sinensis* when concentrations as high as 66 mg m^{-3} have been measured (Vant & Budd 1993); but most years the bloom peaks at about 25–50 mg m^{-3} (Vant unpubl. data).

Samples for identification and enumeration of microzooplankton were also collected from two other contrasting regions of the harbour (Vant & Budd 1993): the similarly turbid, but much less nutrient-enriched south-east, and the clearer, low-nutrient entrance waters.

Experimental procedures

Phytoplankton growth and microzooplankton grazing rates were determined by the dilution technique (Landry & Hassett 1982). Briefly, the technique estimates phytoplankton growth rate and microzooplankton grazing rate by measuring the change in phytoplankton biomass (here measured as changes in chlorophyll *a*) in a series of incubations in which the original water sample has been diluted with filtered water from the same site. Dilution with filtered water reduces the rate of contact between phytoplankton prey and microzooplankton predators, so that the impact of grazing on the net rate of change of phytoplankton is progressively diminished as the proportion of undiluted water in the incubations is reduced. Under the assumption that microzooplankton clearance rate remains constant as the phytoplankton become more dilute (i.e., microzooplankton feeding kinetics are linear), and that microzooplankton biomass remains constant, the observed net specific rate of biomass growth μ_n is given as the solution of the equation for exponential growth as a function of the proportion of undiluted water X:

$$\mu_n(X) = \frac{1}{\Delta t} \ln\left(\frac{B_x(\Delta t)}{XB_i(0)}\right) = \mu - Xg \qquad (1)$$

where $\mu_n(X)$ = net phytoplankton-specific growth rate at dilution X (d^{-1}), Δt = duration of incubation (d), $B_I(0)$ = phytoplankton biomass concentration in the undiluted sample at the start of the incubation—i.e., time = 0 (mg m^{-3}), $B_X(\Delta t)$ = phytoplankton biomass at dilution X and time Δt, μ = phytoplankton-specific growth rate (d^{-1}), and g = microzooplankton-specific grazing rate (d^{-1}). In a series of incubations at a range of values of X, a plot of μ_n versus X should yield a line with slope = $-g$ and intercept = μ.

We conducted incubations at six dilution levels, ranging from $X = 0.05$ to 1.0. The very high dilution ($X = 0.05$) allowed us to determine phytoplankton growth and microzooplankton grazing rates when the assumption of linearity of microzooplankton feeding kinetics was not met. Non-linearity was deemed to occur when the grazing rate at low

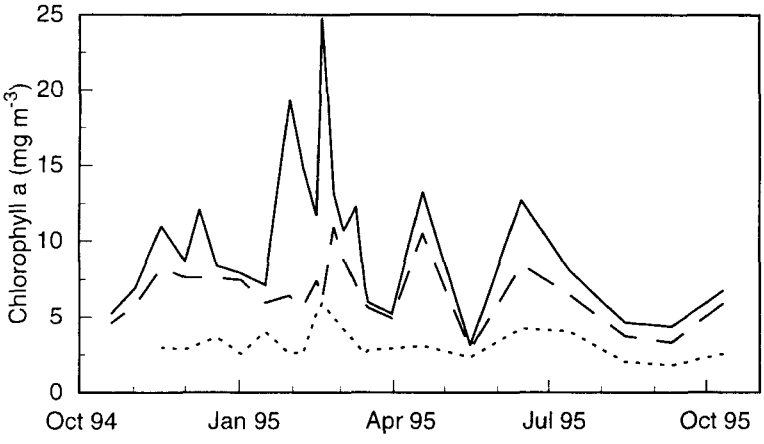


Fig. 1 Concentration of size-fractionated chlorophyll *a* in the north-east region of Manukau Harbour from October 1994 to October 1995. (---) > 0.7 μm (i.e., total); (— —) < 22 μm; (- -) < 5 μm. Distance between lines is a measure of pigment biomass in the intervening size fraction; ticks on the time scale indicate beginning of the month.

dilutions (i.e., $X > 0.4$) was both low (< 0.2 d^{-1}) and a minor proportion (< 40%) of the overall rate. In these instances we used equations 8 and 11 of Gallegos (1989) to estimate μ and g. Microzooplankton net growth rate (μ_Z) was calculated from changes in the biovolume of ciliates in the undiluted treatment. Linear feeding kinetics were also corrected for the growth of grazers during the incubation by dividing linear regression slopes by the relative geometric mean predator density (GMPD, see Appendix 1).

Water samples were collected by bucket from an abandoned harbour bridge. Water for diluent was filtered first through a 125 mm GF/C then through a 47 mm GF/F glass fibre filter. Filtration of the water for diluent required about 2 h because of the high concentrations of suspended solids in Manukau Harbour. To avoid prolonged containment of the undiluted plankton sample we first obtained about 20 litres for filtration, then returned to the site to collect a sample for plankton. This latter sample was collected using a slowly sinking bucket covered with a 200 μm screen to exclude large zooplankton.

Samples at each dilution level were incubated in duplicate 2.4-litre polycarbonate bottles for 24 h at a nearby laboratory. The bottles were placed in a shallow tray outdoors, covered with green gauze that transmitted about 40% of the incident light, and cooled by flowing tapwater. The temperature of the tapwater was within ± 1°C of harbour temperatures and generally stable to within ± 1°C during the incubations. Concentrations of chlorophyll *a* were measured fluorometrically by the method of Strickland & Parsons (1972), using Aminco and Perkin-Elmer fluorometers. Concentrations in the < 5 μm, 5–22 μm and > 22 μm size fractions were determined following Vant & Safi (1996).

Microzooplankton in the undiluted water at the start and finish of each incubation were identified and counted. Subsamples of 50 ml of Lugol's iodine-preserved water were settled before counting at ×100–400 magnification (Lietz Diavert inverted microscope). Ciliate taxa were separated into tintinnids, large oligotrichs (i.e., *Strombidium* sp. and *Strobilidium* sp.), and small oligotrichs (predominantly *Lohmanniella* sp., *Leegardiella* sp., and *Halteriidae*). Identifications were based primarily on Kofoid & Campbell (1929), Tregouboff & Rose (1957), Corliss (1961), and Montagnes & Lynn (1991). Biovolumes of typical members of each taxon observed were determined and the biovolume in each of the three groups calculated. The unfilled portions of the tintinnids' loricae were ignored when determining biovolumes.

RESULTS

Plankton standing stocks

Chlorophyll *a* concentrations in Manukau Harbour varied between 4 and 25 mg m^{-3} during October 1994–October 1995 (Fig. 1). The greatest variability occurred in the > 22 μm size fraction which varied from < 1 to > 15 mg m^{-3} during the bloom of *O. sinensis* in February. The 5–22 μm size fraction varied from < 1 to about 7 mg m^{-3}, and the < 5 μm size fraction varied the least, from about 2 to 6 mg m^{-3} (Fig. 1).

Annual median abundance and biovolume of all three categories of ciliates were higher at the north-east site than at the south-east site or at the harbour

entrance (Table 1). At each site the median biovolume of tintinnids was similar to that of large oligotrichs. At the two inner harbour sites the biovolume of small oligotrichs was about twice that of the other categories, whereas at the harbour entrance the biovolume of small oligotrichs was about half that of large oligotrichs and tintinnids (Table 1). Evidently conditions within the harbour favour growth of small oligotrichs, and the enriched conditions at the north-east site favour all categories.

At times copepod nauplii or polychaete larvae were also present in the 200 μm screened water. Nauplii abundance peaked at the north-east site at c. 1×10^3 l^{-1} in April and May (i.e., autumn), whereas polychaete larvae reached c. 1×10^3 l^{-1} in October 1995. The median abundance of nauplii plus larvae, however, was $< 0.1 \times 10^3$ l^{-1}. Although copepod nauplii have rather higher per capita clearance rates than ciliates (e.g., Parsons et al. 1984: table 24), this was usually offset by the much greater abundance of the ciliates, suggesting they were generally the dominant grazers.

Table 1 Median abundance and biovolume of three groups of microzooplankton at three sites, Manukau Harbour, November 1994–October 1995.

	North-east	South-east	Entrance
Abundance (10^3 individuals l^{-1})			
Tintinnids	1.7	0.5	0.5
Large oligotrichs	1.8	0.3	0.2
Small oligotrichs	9.5	1.8	0.3
Biovolume (10^6 μm^3 l^{-1})			
Tintinnids	11.9	2.6	2.8
Large oligotrichs	14.2	3.2	2.4
Small oligotrichs	36.1	7.0	1.3

Grazing experiments

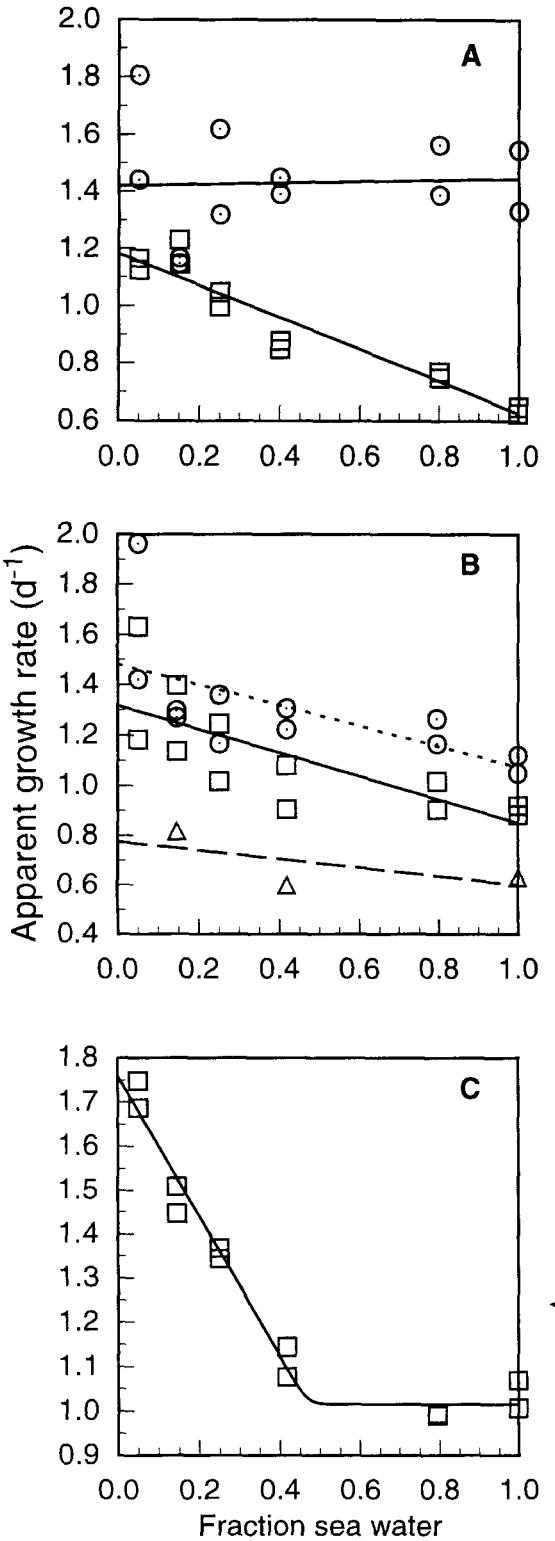
Examples of results obtained in the dilution experiments are shown in Fig. 2. Phytoplankton growth and microzooplankton grazing rates were estimated on the < 5 μm and 5–22 μm size fractions separately on all sampling dates except the first. In October 1994 these fractions were combined (i.e., < 22 μm). As the larger tintinnids—which probably grazed the 5–22 μm size fraction, see below—were not abundant on this occasion, we regard the resulting growth and grazing rates as being estimates of those for the < 5 μm size fraction.

Apparent growth rates were generally less precise in the 5–22 μm size fraction (Table 2), partly because measurement errors were inherently larger for that fraction (which was calculated by subtraction). Only in the February 1995 experiment, during the peak of the *O. sinensis* bloom, were we

Table 2 Correction of phytoplankton growth and grazing mortality rates (μ and g, respectively) for growth of microzooplankton during dilution experiments (growth rate μ_Z), and when present, non-linearity of microzooplankton feeding kinetics. Values in brackets were not significantly different from zero ($P < 0.05$), and were not corrected. Type of correction: G, geometric mean predator density (correcting g only); N, non-linear feeding kinetics (correcting μ and g). Units of all rates are d^{-1}.

		< 5 μm phytoplankton					5–22 μm phytoplankton				
		Regression		Corrected			Regression		Corrected		
Month	μ_Z	μ	g	Type	μ	g	μ	g	Type	μ	g
Oct 94	0.49	1.14[a]	0.22[a]	N	1.24[a]	0.35[a]	–	–	–	–	–
Nov 94	0.60	0.89	1.44	G	0.89	1.07	0.40	(–0.07)	–	0.40	–
Jan 95	0.14	1.18	0.57	G	1.18	0.53	1.42	(–0.02)	–	1.42	–
Feb 95	0.18	1.32	0.44	N	1.45	0.72	1.48	0.40	G	1.48	0.37
Mar 95	–0.23	1.59	0.66	N	1.83	1.27	1.23	(0.10)	–	1.23	–
Apr 95	0.82	1.37	0.71	G	1.37	0.47	0.33	(0.08)	–	0.33	–
May 95	0.31	0.55	0.92	G	0.55	0.79	1.75	0.90	G	1.75	0.77
Jun 95	0.30[b]	0.16	0.43	G	0.16	0.37	(0.02)	(–0.19)	–	–	–
Jul 95	0.28	0.29	0.47	G	0.29	0.41	0.44	(–0.08)	–	0.44	–
Aug 95	0.20	0.50	0.64	N	0.82	0.80	0.25	(–0.08)	–	0.25	–
Sep 95	0.55	0.62	0.41	G	0.62	0.31	0.58	(0.02)	–	0.58	–
Oct 95	0.12	0.51	0.46	N	0.74	0.63	0.19	0.17	G	0.19	0.16

[a]Estimated from < 22 μm fraction (see text)
[b]No sample for microzooplankton counts at end-of-incubation; value is mean of measured rates for other months (including Dec 94 when μ_Z was 0.20 d^{-1})



able to determine apparent growth rates for the > 22 μm size fraction (Fig. 2B). To do so it was necessary to conduct additional incubations using water that had not been screened to remove large zooplankton. Growth and grazing rates in that size fraction were lower than in the smaller size fractions (Fig. 2B).

On several occasions we observed non-linear growth response curves (Fig. 2C, Table 2), indicative of non-linear feeding kinetics of the microzooplankton. The corrected phytoplankton growth rates were 10–65% higher than the estimates based on linear regression, whereas the microzooplankton grazing rates were 25–90% higher (Table 2). The GMPD correction (Appendix 1) for microzooplankton growth and linear grazing kinetics does not affect the linear regression estimates of phytoplankton growth rate, but corrected grazing rates were 6–34% lower than the uncorrected estimates (Table 2), depending on the magnitude of μ_Z.

Plankton dynamics

Phytoplankton growth rates in the < 5 μm size fraction varied seasonally, from a minimum of about 0.2 d^{-1} in June to 1.8 d^{-1} in March (Fig. 3A). Growth rates in the 5–22 μm size fraction varied over a similar range as the < 5 μm size fraction. The minimum growth rate in the 5–22 μm size fraction occurred in October, but the maximum, 1.75 d^{-1}, surprisingly occurred in May. Aside from the anomalously high rate in May, highest growth rates in the 5–22 μm size fraction, 1.2–1.5 d^{-1}, were observed in the summer months (Fig. 3A). The summertime growth rates, which were measured without addition of nutrients, are about as high as expected for nutrient-saturated rates at 20°C (Eppley 1972; Banse 1982), which suggests that the experimental irradiance regime and the ambient nutrient concentrations were saturating to growth.

◀ **Fig. 2** Examples of results obtained in dilution experiments at Manukau Harbour. **A**, January 1995: Growth and grazing rates could be discerned for the < 5 (□) and the 5–22 (○) μm size fractions; **B**, February 1995: During the bloom of *O. sinensis* growth and grazing rates were also measurable on the > 22 μm size fraction (Δ). **C**, March 1995: Non-linear growth response curves indicative of nonlinear feeding kinetics were occasionally observed. Fitted curve is based on type I (piecewise linear) functional response.

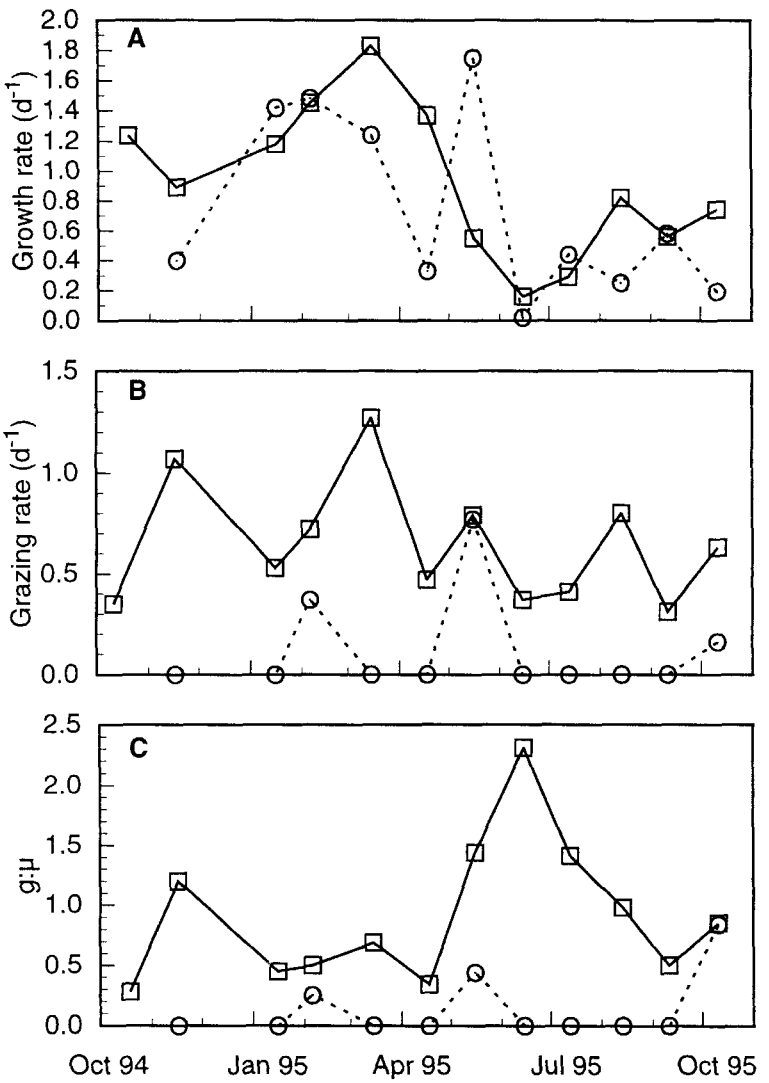


Fig. 3 **A**, Seasonal variation in growth rates of Manukau Harbour phytoplankton in the size fractions; **B**, Seasonal variation in microzooplankton grazing rate; **C**, Ratio of microzooplankton grazing rate to phytoplankton growth rate. □, < 5 μm; ○, 5–22 μm.

Microzooplankton grazing rates varied from 0.3 to 1.3 d^{-1} on the < 5 μm size fraction and from 0 to 0.8 d^{-1} on the 5–22 μm size fraction (Fig. 3B). In May, grazing rates in the two size fractions were nearly equal, but otherwise grazing rates in the 5–22 μm size fraction were low (< 0.4 d^{-1}, or only slightly higher than the minimum for the < 5 μm fraction), and were often 0 d^{-1} (Fig. 3B). The mean grazing rates for the < 5 μm and 5–22 μm size fractions were 0.6 and 0.1 d^{-1}, respectively. In February and May relatively rapid grazing of the 5–22 μm size fraction coincided with peaks in the abundance of tintinnids. In May, copepod nauplii were also relatively abundant, but they were not observed in February. By contrast, although nauplii were also abundant in April, and tintinnids were abundant in June and September, grazing on the 5–22 μm size fraction was low on these occasions.

The ratio of grazing rate to phytoplankton growth rate varied from about 0.3 to 2.3 (Fig. 3C). In the < 5 μm size fraction, grazing rate usually exceeded (or was nearly equal) growth rate from May through October. During summer the ratio of $g:\mu$ was fairly steady at about 0.5 for the < 5 μm size fraction. In the 5–22 μm size fraction the ratio of $g:\mu$ was nearly 1 in October 1995, but was

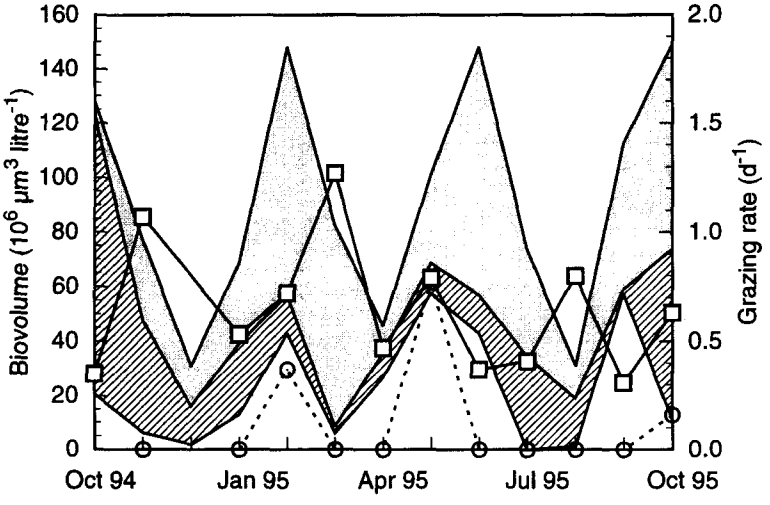


Fig. 4 Seasonal variation in biovolume of microzooplankton categories at the north-east site: tintinnids (clear); large oligotrichs (hatched); small oligotrichs (shaded); superimposed are microzooplankton grazing rates on the < 5 (□) and 5–22 µm (○) phytoplankton size fractions.

otherwise less than about 0.5 (Fig. 3C), and often 0 owing to absence of grazing on that size fraction. On average about 90% of the production of the < 5 µm size fraction was grazed, compared to less than 20% of the 5–22 µm size fraction.

Biovolumes of the three categories of microzooplankton were highly variable at the north-east site (Fig. 4). Each category at one time or another dominated the microzooplankton biovolume. No clear relationship existed between the grazing rate on the < 5 µm size fraction and any of the three categories of microzooplankton. The two highest grazing rates for the 5–22 µm size fraction coincided with peaks in the biovolume of tintinnids in February and May (Fig. 4); but the correspondence was not consistent, as we observed insignificant grazing on the 5–22 µm size fraction in June and September when tintinnids were of similar abundance as in February and May.

DISCUSSION

Size-dependence of grazing rates

It can be shown that if the assumptions of the dilution technique hold for each component population in a mixed assemblage, then the aggregate growth and grazing rates estimated from changes in chlorophyll *a* are functions of the component growth and grazing rates, strongly weighted (but not uniquely determined) by the initial biomass of each component. Thus a segment of the total community that comprises a large fraction of the biomass, but which has a low grazing loss rate, may partially mask the influence of component which is less abundant, but turning over rapidly.

Previous attempts to disaggregate the overall grazing rate into components, e.g., analysis of class-specific pigments by high-performance liquid chromatography (HPLC) (Burkhill et al. 1987; Welschmeyer et al. 1991), flow cytometry of picoplankton with differing fluorescence signatures (Landry et al. 1995), and microscopy (Landry et al. 1984), have yielded useful insight into the dynamics of the microplankton community. For example, Welschmeyer et al. (1991) determined that smaller, carotenoid-containing phytoplankton grew at moderate rates that were matched by microzooplankton grazing, whereas faster-growing fucoxanthin-containing cells (diatoms) were relatively ungrazed by microzooplankton, and formed blooms inside incubation bottles towards the end of the experiments.

Size-fractionation of initial and final chlorophyll samples enabled us to estimate growth and grazing rates on the < 5 and 5–22 µm size fractions separately (Fig. 2, 3). Size-fractionation is especially suited to estuarine and coastal areas where phytoplankton too large for flow cytometry may be abundant, and where, as in Manukau Harbour, suspended sediment loads preclude filtration of sufficient volumes for HPLC analysis. Similar to the results of Strom & Strom (1996), who used an 8-µm filter, high rates of grazing were sometimes observed in both size fractions (Fig. 3B).

Except on two dates, however, the grazing rate on the 5–22 μm size fraction in Manukau Harbour was less than c. 0.3 (often 0) d^{-1}, and was generally < 20% of the growth rate in that size fraction (Fig. 3B, 3C). The two instances of high grazing rates on the 5–22 μm size fraction coincided with high biovolumes of tintinnids (Fig. 4). Thus size-fractionation may provide useful information on trophic pathways in the microbial food web.

Impact of microzooplankton grazing *in situ*

Grazing by microzooplankton was always measurable on the < 5 μm size fraction in Manukau Harbour, but only sporadically so on the 5–22 μm size fraction. Grazing as a fraction of growth rate in the < 5 μm size fraction varied from about 0.5 in summer to > 1 much of the winter (Fig. 3C). Clearly then, microzooplankton grazing is capable of preventing formation of blooms by small size fractions during the winter and spring. However, g cannot exceed μ for months at a time. We suspect that our monthly sampling missed times in the winter when μ exceeded g.

Whether the observed microzooplankton grazing rates are sufficient to prevent bloom formation during the summer, when the larger size fractions increase dramatically, remains unsettled. Our incubation procedure measured growth rates at saturating light intensities. If in situ growth rates were also maximal (e.g., 1.4 d^{-1}: Fig. 3A), and grazing were the only significant loss process, then the residual net growth rates of about 0.6 d^{-1} would be sufficient to produce substantial blooms in the < 5 μm size fraction. Thus either the realised growth rates in the turbid, well-mixed harbour were less than maximal, or additional loss processes occurred to reduce in situ net growth rates of the < 5 μm size fraction. To resolve this question we consider realised in situ growth rates and additional loss processes in the context of a mathematical model (McBride et al. 1993) of the north-east arm of Manukau Harbour.

McBride et al. (1993) constructed a mathematical model of light-limited phytoplankton growth in Manukau Harbour, similar to that of Cloern (1991) for San Francisco Bay. The model is 2-dimensional (depth and time) and includes processes of photosynthesis, respiration, zooplankton grazing, benthic filtering, settling, and vertical and horizontal exchange with the coastal boundary. Light attenuation is dependent on suspended particulate matter driven by measured wind speed and tidal scour, and vertical mixing depends on tidal amplitude. McBride et al. (1993) obtained broad agreement between measured and simulated chlorophyll concentrations using plausible parameter values and measured meteorological functions for the spring-summer of 1991/92, a year with a remarkably large bloom of *O. sinensis*. Here we use the model to define a plausible in situ light environment and horizontal exchange rates for the < 5 μm size fraction, in order to examine the impact of the measured grazing rates on physically constrained phytoplankton growth rates.

To fit the chlorophyll concentrations measured during spring through autumn 1991–92, McBride et al. (1993) used "species-dependent" biological parameters based on observed changes in phytoplankton species composition. Predicted levels were especially sensitive to the carbon: chlorophyll ratio, assumed to be high in the spring

Table 3 Values of biological parameters used in modelling total phytoplankton chlorophyll by McBride et al. (1993) and modifications made here to model < 5 μm size-fraction in Manukau Harbour. P_m^B chlorophyll-specific light-saturated photosynthesis rate.

Parameter/ process	Value calibrated for 1991–92	Modified value for < 5 μm fraction	Units	Source/justification
Respiration	0.10[a] to 0.35[b]	0.15	Proportion of P_m^B	Langdon (1993)
Sinking velocity	0.5[b] to 5.0[a]	0	m d^{-1}	Small and flagellated cells
Grazing loss rate	0–0.1	0.6	d^{-1}	Present study
C:Chla ratio	34[a] to 106[b]	34	mg C (mg Chla)$^{-1}$	Small diatoms in McBride et al. (1993); see also Gallegos & Vant (1996)

[a]During bloom of *Odontella sinensis*
[b]During period of dinoflagellate dominance

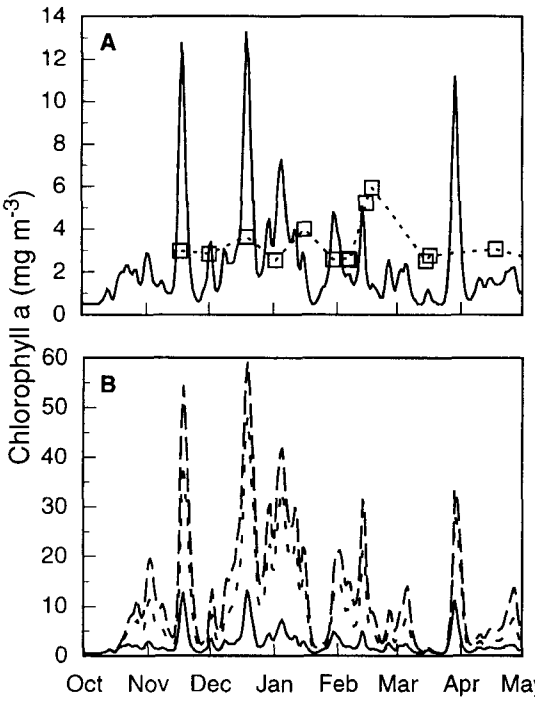


Fig. 5 **A**, Chlorophyll *a* concentrations measured in Manukau Harbour during spring through autumn 1994/95 in the < 5 μm size fraction (squares) and those predicted by a 2-dimensional advective diffusion model of phytoplankton growth driven by wind speed and sunlight data from 1991/92 (solid line). Parameters used in the simulation represented assumed biological attributes of < 5 μm size fraction, and microzooplankton grazing rate similar to that measured in January and February 1995 (see Table 3). **B**, Sensitivity of simulated chlorophyll *a* concentration to variation in grazing rate. Solid line: $g = 0.6$ d^{-1}; short dashes: $g = 0.1$ d^{-1}; long dashes: $g = 0$ d^{-1}. Solid line is same curve as in A.

during a period of observed dinoflagellate dominance, and to settling velocity, which was assumed to be high during the period when *O. sinensis* bloomed and low all other times. Here we altered selected biological parameters to represent physiological characteristics of the < 5 μm size fraction of phytoplankton (Table 3), and used a grazing rate based on our measured values (Fig. 3B) to simulate expected chlorophyll concentrations in the < 5 μm size fraction for spring through autumn growing season.

Predicted chlorophyll concentrations were highly variable owing to the dependence of light attenuation on wind speed; but predicted concentrations fluctuated above and below the 2–5 mg m^{-3} observed in the < 5 μm size fraction during November 1994–April 1995 (Fig. 5A). Predicted chlorophyll concentrations were highly sensitive to g (Fig. 5B). Reduction of g to 0.1 d^{-1} resulted in predicted chlorophyll concentrations similar to total chlorophyll, i.e., much greater than those observed in the < 5 μm size fraction. We conclude that the measured grazing rates on the < 5 μm size fraction are sufficient to prevent them from attaining bloom levels of the magnitude observed for *O. sinensis* in Manukau Harbour. Furthermore, light-limitation appears to cause average in situ growth rates to be about half the maximal rates.

More intriguing is the frequent absence of grazing of the 5–22 μm phytoplankton. This is a size range that should not necessarily confer a refuge from microzooplankton grazing, especially when considering the variety of feeding modes prevalent among, e.g., heterotrophic dinoflagellates (Morey-Gains & Elbrachter 1987; Strom & Strom 1996). In Manukau Harbour, heterotrophic dinoflagellates were uncommon and did not occur frequently enough to warrant a counting category (Table 1). The occasional moderate-to-high grazing rates on the 5–22 μm size fraction coincided with peaks in the abundance of tintinnids (February and May), copepod nauplii (May), or polychaete larvae (October 1995); but at other times peaks in the abundance of tintinnids or nauplii were associated with low grazing rates. The factors controlling the 5–22 μm size fraction in the harbour at times when microzooplankton grazing is low remain to be determined; grazing by zooplankton and benthic bivalves is probably important (e.g., Cloern 1982; Berggreen et al. 1988).

Vant & Safi (1996) showed that at any given time of the year the different-sized phytoplankton in the north-east part of the harbour all grew at similar rates. The failure of the smaller size fraction to bloom was attributed to their faster removal from the water column. We have now shown that cells of the < 5 μm size fraction at least are indeed rapidly removed by heavy grazing by the abundant microzooplankton.

For these smallest phytoplankton, both the light-saturated growth rates measured in this study (Fig. 3A) and the integral rates through the euphotic zone (Vant & Safi 1996: fig. 6) varied seasonally, with highest values—and thus highest production—occurring during the summer. By contrast, neither the abundance nor the grazing rate of the microzooplankton showed any clear seasonal pattern (Fig.

3B, Fig. 4). This suggests that during the winter in particular the microzooplankton may be supported by alternative food sources; one possibility is the dead and dying (freshwater) phytoplankton and bacterioplankton which are discharged at high levels from the wastewater treatment plant (Vant & Budd 1993). The presence of this detrital material in the north-east harbour may explain the substantially larger population of microzooplankton found there (Table 1), even though average levels of phytoplankton production are no higher than in other parts of the harbour (Vant & Budd 1993). Furthermore, greater amounts of detrital material at both inner harbour sites may enhance bacterial activity, selectively stimulating the growth of small (bacterivorous) oligotrichs. The absence of a clear relationship between grazing on < 5 μm phytoplankton and microzooplankton biovolume would, then, be expected.

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APPENDIX 1

As discussed by Landry (1993), the dilution technique attempts to measure the grazing mortality rate of phytoplankton by progressively reducing the effect of grazing on phytoplankton growth in a series of dilutions. A stated assumption for using Xg as a surrogate for the relative grazing loss is that microzooplankton biomass remains constant during the incubation (see above, Experimental procedures). In fact, microzooplankton growth rates are comparable to those of their phytoplanktonic prey (Banse 1982), so that there is a potential for the relative increase in density of microzooplankton to equal or exceed that of the phytoplankton, which would violate the assumption of constant microzooplankton biomass. The problem can be approached quantitatively by examining the coupled equations for phytoplankton and microzooplankton growth (Gallegos 1989):

$$\frac{dB}{dt} = B(\mu - FZ) \tag{A1a}$$

$$\frac{dZ}{dt} = Z(\psi FB - m_z) \tag{A1b}$$

where Z = microzooplankton biomass density, F = volume swept clear per unit microzooplankton biomass per unit time, ψ = gross growth efficiency, m_Z = microzooplankton mortality rate, and B and μ were defined previously. We make the assumption that F is constant over the range of B of interest, thereby restricting interest to the situation of linear microzooplankton feeding kinetics.

The grazing rate in Eqn A1a is given by $g = F Z$, so that whenever $\psi F B \neq m_Z$, Z and hence also g will change during the course of an incubation. Because the initial density of phytoplankton is altered by dilution, the microzooplankton net growth rate, $\psi F B - m_Z$, will vary among the different dilution treatments. In general it is the value of g in the undiluted treatment at the time of sampling and experiment setup that is the quantity of interest. Landry (1993) suggested that apparent growth rate be regressed against the relative mean predator density (MPD) as the independent variable to correct for microzooplankton growth. MPD is given by

$$MPD = \frac{Z_0}{\mu_z \Delta t}\left(e^{\mu_z \Delta t} - 1\right) \tag{A2}$$

where Z_0=initial microzooplankton density
and $\mu_Z = \ln(Z_{\Delta t}/Z_0)$ is the average net growth rate of the microzooplankton. Relative MPD is calculated by dividing Eqn A2 by Z_0.

Because of the multiplicative interaction between B and Z in Eqn A1a, and because of the exponential growth of Z when $\mu_Z > 0$, we considered that the geometric mean predator density, GMPD, might be a superior transformation for recovering the true initial grazing rate in dilution experiments. GMPD is given by

$$GMPD = \left[Z_0 Z_{\Delta t}\right]^{1/2} = Z_0 e^{\mu_z \Delta t / 2} \tag{A3}$$

and dividing Eqn A3 by Z_0 gives relative GMPD as simply $e^{\mu_z \Delta t / 2}$.

We conducted simulations of the coupled set of Eqn A1a and A1b using plausible values for parameters ψ, F, and m_Z, and initial conditions B_0 and Z_0 (in arbitrary biomass units) selected to give a range of μ_Z and g (Table A1). For each set of parameters we simulated a dilution experiment by conducting seven integrations of Eqn A1a and A1b, sequentially multiplying the initial values B_0 and Z_0 by the factor X = 1, 0.8, 0.6, 0.2, 0.1, and 0.05. In a few simulations we fixed μ_Z arbitrarily and held it constant at all values of X, rather than allowing it to vary with prey density (and hence also with X) as in most simulations. Apparent growth rates were calculated in the usual way, and regressed against

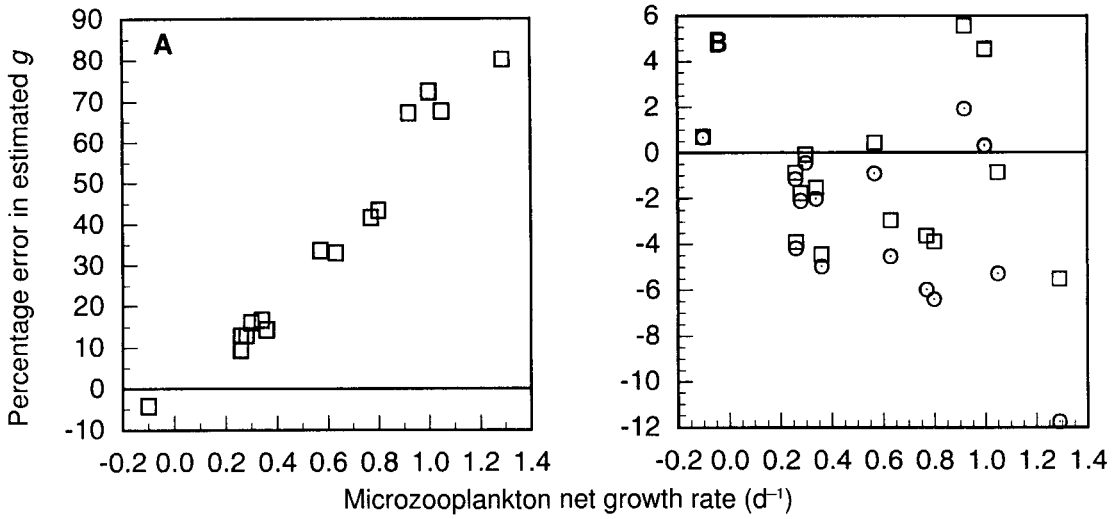

Fig. A1 **A**, Relative error in estimated grazing rate from simulations of equations A1, as a function of microzooplankton net growth rate in undiluted sample, $\mu_Z(1.0)$. Estimated g was not corrected for growth of microzooplankton. **B**, Relative error in estimated grazing rate after correcting for ○, relative mean predator density (MPD), and □, relative geometric mean predator density (GMPD) as a function of microzooplankton net growth rate in undiluted incubation.

X without correction, with relative MPD correction, and relative GMPD correction. Percentage error was calculated as 100 $(\hat{g}-g)/g$, where $\hat{g}$ is the value estimated by linear regression with or without one of the corrections.

Failure to correct for microzooplankton growth in the simulated dilution experiments resulted in serious errors in estimated grazing rates (Fig. A1A). The errors increased in magnitude with increasing μ_Z. Grazing rate was overestimated by as much as 80% for $\mu_Z \geq 1$ d^{-1}. Division of the slopes by relative MPD or relative GMPD reduced the errors to a range from +6 to −12% (Fig. A1B). In all but three of the simulations the GMPD correction was superior to the MPD correction. The improvement ranged from negligible to about 7%. Two of the three instances in which the MPD correction was superior to GMPD correction were simulations in which microzooplankton growth rate was uncoupled from consumption (i.e., μ_Z was arbitrarily fixed at all values of X). Because we deem this an unrealistic situation, and because the GMPD correction was slightly superior, we used it to correct for growth of microzooplankton in our field experiments.

Table A1 Values of parameters used in simulation of set of equations for coupled phytoplankton growth and microzooplankton growth and grazing. ψ (dimensionless), gross growth efficiency; F (volume swept clear biomass^{-1} d^{-1}), microzooplankton clearance rate; Z_0 (arbitrary biomass concentration), initial microzooplankton density; m_Z (d^{-1}), microzooplankton mortality rate; g (d^{-1}), microzooplankton grazing rate (i.e., FZ_0); $\mu_Z(1.0)$ (d^{-1}), microzooplankton net growth rate in undiluted water; $\mu_Z(0.05)$ (d^{-1}), microzooplankton net growth rate in 95 percent diluted (i.e., fraction unfiltered water, X=0.05); $\hat{g}$ (d^{-1}), grazing rate estimated without correction for microzooplankton growth during incubation. Microzooplankton growth was uncoupled from consumption of phytoplankton for selected simulations (–).

ψ	F	Z_0	m_z	g	$\mu_Z(1.0)$	$\mu_Z(0.05)$	$\hat{g}$
0.6	0.25	2.0	0.1	0.50	0.63	–0.05	0.665
0.3	0.25	2.0	0.1	0.50	0.28	–0.07	0.565
0	0.25	2.0	0.1	0.50	–0.10	–0.10	0.479
–	0.25	2.0	–	0.50	0.30	0.30	0.581
–	0.25	2.0	–	0.50	1.00	1.00	0.862
0.6	0.25	2.0	0.4	0.50	0.36	–0.35	0.572
0.6	0.30	3.0	0.1	0.90	0.57	–0.04	1.202
0.3	0.30	3.0	0.1	0.90	0.26	–0.07	1.016
0.6	0.25	1.0	0.1	0.25	0.77	–0.05	0.354
0.3	0.25	1.0	0.1	0.25	0.34	–0.07	0.292
0.6	0.10	2.5	0.1	0.25	0.26	–0.01	0.234
–	0.30	3.0	–	0.90	1.00	1.00	1.551
–	0.25	1.0	–	0.25	1.00	1.00	0.430