

Growth and grazing within the microbial food web of a large coastal embayment

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ABSTRACT: The complex role of the microbial food web was investigated through a series of size fractionated grazing experiments conducted in the north-east of Manukau Harbour, a large shallow coastal embayment in the Auckland region, New Zealand. The harbour site chosen for this study is considered eutrophic, with total inorganic nitrogen levels generally exceeding 35 μM and soluble reactive phosphorus levels varying between 3 and 6 μM . A late summer bloom of large diatoms ($>22 \mu\text{m}$) is a regular feature and results in chlorophyll *a* (chl *a*) concentrations between 20 and 60 mg m^{-3} . For the rest of the year, mean chl *a* concentrations are around 5 mg m^{-3} and phytoplankton cells are generally $<22 \mu\text{m}$. Microzooplankton grazing on phytoplankton and heterotrophic prey was investigated in 2 experiments which were run simultaneously at monthly intervals on water collected from this site. These experiments were able to identify the separate grazing impacts of $<22 \mu\text{m}$ and 22 to 200 μm microzooplankton. The $<22 \mu\text{m}$ microzooplankton grazer population was dominated by heterotrophic nanoflagellates (HNF) and was responsible for the majority of grazing on organisms $<5 \mu\text{m}$ in diameter. Results from these experiments indicated that, on average, HNF alone consumed all the prokaryotic picophytoplankton production, 87% of the bacteria production and 75% of the eukaryotic $<5 \mu\text{m}$ phytoplankton production. Heterotrophic prey in the form of bacteria was the dominant prey in the $<5 \mu\text{m}$ size fraction throughout the year, with HNF consuming an average of 1.9 times as much bacterial biomass as phytoplankton biomass. The $<200 \mu\text{m}$ microzooplankton population was dominated by ciliates and grazed 37% of the 5 to 22 μm chl *a* weight specific production. These grazers were also partially sustained by feeding on heterotrophic food sources, with 63% of the HNF production being grazed.

KEY WORDS: Microzooplankton · Nanoflagellates · Ciliates · Grazing · Dilution experiments

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INTRODUCTION

Recent evidence suggests that the microbial food web is a key component in coastal and estuarine ecosystems (Vaqué et al. 1992, Jimenez-Gomez et al. 1994). Within the microbial food web, protozoa $<20 \mu\text{m}$ are recognised as the primary grazers of bacteria and picophytoplankton (Davis & Sieburth 1986, Bernard & Rassoulzadegan 1990, Capriulo 1991). These protozoans are often in turn the prey of larger organisms,

which may also consume nanophytoplankton ($<20 \mu\text{m}$) and microphytoplankton ($<200 \mu\text{m}$) (Riegman et al. 1993).

The organisms responsible for grazing within the microbial web can be collectively referred to as microzooplankton, which can be operationally defined as mixotrophic and heterotrophic organisms that pass through a 200 μm screen (Gallegos et al. 1996). It is generally considered that the high growth rate of microzooplankton should enable them to control assemblages of bacterioplankton and small phytoplankton, at least when temperature does not limit their growth and grazing by metazoans is low (Havens 1993, Riegman et al. 1993).

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In temperate estuarine waters, seasonal shifts in the composition and size distribution of the phytoplankton population occurs throughout the year, and in nutrient-enriched environments phytoplankton blooms are a common feature (Vant & Budd 1993). Manukau Harbour is a large shallow coastal embayment located in the Auckland region, New Zealand, in which chlorophyll *a* (chl *a*) concentrations vary seasonally throughout most of the harbour from around 1 to 15 mg m⁻³. In most years, a late-summer bloom of the large diatom *Odontella sinensis* has been reported in the north-east end of the harbour. This can result in a chl *a* concentration of up to 60 mg m⁻³ in this region, although a concentration of 25 to 30 mg m⁻³ has been more regularly observed (Gallegos et al. 1996). These blooms are supported by elevated nutrient concentrations at this site, resulting from the discharges of an adjacent wastewater treatment plant. Total inorganic nitrogen in this region generally exceeds 35 µM, while soluble reactive phosphorus levels vary between 3 and 6 µM throughout the year (Vant & Budd 1993).

Previous studies in Manukau Harbour have shown that phytoplankton in all size classes grow at similar rates and that for most of the year <22 µm size classes dominate the phytoplankton biomass. However, during the late summer phytoplankton bloom, the larger >22 µm size classes have always been found to dominate (Gallegos et al. 1996, Vant & Safi 1996). Grazing experiments conducted by Gallegos et al. (1996) suggested that microzooplankton grazing might be sufficient to prevent bloom formation by the <22 µm size class.

Gallegos et al. (1996) also reported that microzooplankton numbers were elevated at the north-east harbour site in both summer and winter compared to other areas in the harbour and that neither microzooplankton grazing loss rates nor abundance showed a clear seasonal pattern. In addition, they proposed that heterotrophic food sources might support the high microzooplankton biomass. They went on to suggest that the food web structure supporting this could be based on higher concentrations of detrital material at the north-east site stimulating bacterial production, which in turn may selectively stimulate the growth of small bacterivorous ciliates (oligotrichs).

The aim of this study was to investigate seasonal changes in the microbial food web structure and dynamics by conducting grazing experiments in the north-east corner of Manukau Harbour over the period of 1 year. By determining the grazing pressure not

only on autotrophic (photosynthetic) prey but also on heterotrophic prey in the form of bacteria and heterotrophic nanoflagellates, we aimed to investigate the role of heterotrophic organisms as prey and predators in the food web.

MATERIALS AND METHODS

Study site. Manukau Harbour (37°S, 174°E) is a large (ca. 370 km²) shallow (mean depth ca. 6 m), turbid macrotidal estuary located west of Auckland in the North Island of New Zealand. Monthly samples for this study were collected at high tide from an abandoned harbour bridge in the region, previously designated as the north-east corner (Fig. 1). The water column in this area was generally well mixed by strong tidal currents, with a mean tidal range of 2 to 3.4 m and salinity ranging between 28 and 33‰.

Growth and grazing calculations. Estimates of phytoplankton growth rates and microzooplankton grazing loss rates were made by measuring changes in biomass in a series of incubations in which the original water sample was diluted with filtered water from the same site following the methods described in Landry & Hassett (1982). Following these methods, linear re-

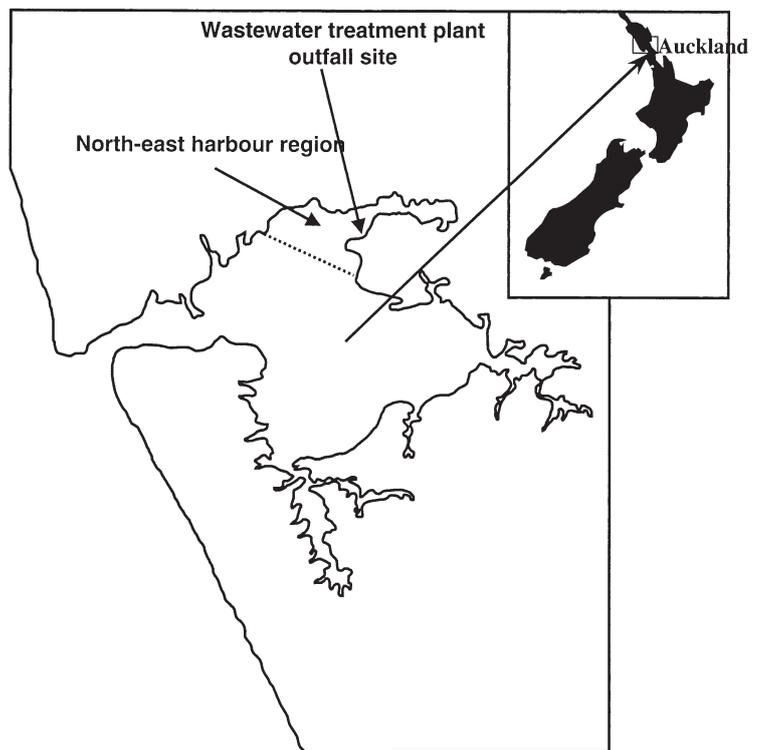


Fig. 1. Sampling site in Manukau Harbour. (.....) indicates the north-east harbour region

sponse curves were only accepted on occasions when the slope had an $R^2 > 50\%$. Nutrient concentrations in this study were always above $5 \mu\text{M}$ for nitrogen and $3 \mu\text{M}$ for phosphorus, which is not considered limiting for phytoplankton growth (Gallegos et al. 1996). When the assumption of linearity was not met as required for the methods described in Landry & Hassett (1982), an alternative calculation of growth and grazing rate was applied. This followed the methodology outlined in Gallegos (1989). These calculations were made possible by the inclusion of a very high dilution treatment (0.05). Calculations for non-linear grazing were applied only when the grazing rate at low dilutions (i.e. >0.4) was both low ($<0.2 \text{ d}^{-1}$) and a minor proportion ($<40\%$) of the overall rate. In these instances, we used Eqs. (8) & (11) of Gallegos (1989) to estimate μ (growth) and g (grazing). 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) following the method described in Gallegos et al. (1996).

Gallegos et al. (1996) found that failure to correct for microzooplankton growth in simulated dilution experiments resulted in serious errors in estimated grazing rates. The errors increased in magnitude with increasing growth rate. Grazing rate was overestimated by as much as 80%. Division of the slopes by relative GMPD reduced the errors to a range from +6 to -12%.

Experimental procedures. Samples were collected at monthly intervals between October 1996 and October 1997. A slowly sinking bucket covered with a $200 \mu\text{m}$ screen was used to collect surface harbour water for grazing experiments. Samples were always collected at approximately the same time of day and always at high tide. The water was then transported to the laboratory within 2 h and kept at ambient temperature. Sub-samples were further screened (fractionated) at $22 \mu\text{m}$ by a slowly sinking tube 100 mm in diameter covered at one end by $22 \mu\text{m}$ mesh.

Harbour water for dilution was filtered at $0.2 \mu\text{m}$. This was achieved by filtering through a 125 mm GF/C then a 125 mm $0.2 \mu\text{m}$ cellulose acetate filter and finally through a 47 mm $0.2 \mu\text{m}$ cellulose acetate filter. The use of a second $0.2 \mu\text{m}$ filter was to insure the removal of all organisms as a small amount of leakage occurred with the 125 mm filtration unit. Filtrations were conducted under low vacuum to minimise cell damage. Filtration of the dilution water required about 2 to 3 h because of the small pore size and the high concentrations of suspended solids in Manukau Harbour.

In a set of 8 acid-washed, 2.4 l polycarbonate bottles, the $<200 \mu\text{m}$ screened water was then diluted with $0.2 \mu\text{m}$ filtered water to concentrations of ~5, 20, 40 and 100% (i.e. undiluted). These experiments were

designed to measure total microzooplankton grazing. Simultaneously, in a second set of 8, 300 ml glass bottles the $<22 \mu\text{m}$ screened water was diluted with $0.2 \mu\text{m}$ filtered water at the same dilution levels. This second set of experiments ($<22 \mu\text{m}$) was conducted to measure grazing by nanoplankton. Incubations for all experiments were conducted in duplicate bottles for 24 h.

The total standing time between water collection and the start of the experiments was 4 to 5 h. No significant changes were observed in the population structure of the biological organisms measured in these experiments over the preparation period. Trials involving dual experiments were conducted. One experiment was conducted very near the collection site and another experiment 1 h driving distance away. It was found that holding the water for an extra hour had no significant effect on the experimental results (authors' unpubl. results). All bottles were then placed in a shallow tray outdoors, cooled by flowing tapwater and covered with shade cloth that transmitted ~40% of the incident light, simulating harbour conditions. The temperature of the tapwater was within $\pm 1^\circ\text{C}$ of ambient harbour temperature.

The abundance of microzooplankton and other heterotrophic organisms was determined by direct microscope counts in both sets of experiments. Sub-samples were taken for analysis of ciliates, heterotrophic nanoflagellates (HNF) and bacteria (Hpicos). The presence or absence of other microzooplankton was recorded with copepod naupii, polychaete larvae and dinoflagellates being observed. The abundance and biomass of these groups were low and they were excluded from further analysis.

Microscopic examination of the <200 and $<22 \mu\text{m}$ screened water for microzooplankton revealed that ciliates dominated the 22 to $200 \mu\text{m}$ size fraction in terms of both biomass and numbers, HNF and autotrophic (chl *a*) bearing nanoflagellates ANF dominated the $<22 \mu\text{m}$ size fraction in terms of numbers and biomass. The $<200 \mu\text{m}$ screened water experiment measured grazing by the total microzooplankton population, while the $<22 \mu\text{m}$ screened water experiment measured grazing by nanoplankton. By subtracting the results of the nanoplankton experiment from the microzooplankton experiment, we were able to assess the relative impact of grazers in the size class 22 to $200 \mu\text{m}$ (ciliates).

To assess grazing on the phytoplankton population, sub-samples for size fractionated (<200 , <22 and $<5 \mu\text{m}$) chl *a* were taken from the 'total microzooplankton grazing experiments' (Total). In both Total and 'Nanoplankton grazing experiments' (Nano), sub-samples were taken to determine the composition of $<5 \mu\text{m}$ phytoplankton for comparison with size fractionated

<5 µm chl *a* results. Autotrophic cells <5 µm were classified into 3 groups. Firstly, the <5 µm prokaryotic phytoplankton (Ppicos) which, although dominated by *Synechococcus* sp. (90 to 98%), also included some small benthic genera, preventing this group from being described solely as *Synechococcus* species. All cells reported in this population were determined by orange fluorescence under green light excitation as described in Hall (1991). The second group was <5 µm eukaryotic phytoplankton (PpicosE). This group represented nonflagellated forms (mainly small diatoms) and were identified by red chlorophyll fluorescence as described by Hall (1991). The third group was small autotrophic (chl *a* bearing) flagellate cells <5 µm (<5 µm AF). All ANF in the size class <22 µm were also assessed for comparison with the results of size fractionated <22 µm chl *a* grazing experiments.

Sub-samples for size fractionated chl *a*, <5 µm AF, ANF, Ppicos, PpicosE, HNF and Hpicos were taken during the Total grazing experiments, at the beginning of the experiment (T_0) and 24 h later (T_{24}) in all dilutions, to determine grazing and growth rates. Additional samples for ciliate populations were taken from undiluted water (100%) only at T_0 and T_{24} to determine growth rates. In the Nano grazing experiments, only the smaller cell size populations were sampled with <5 µm AF, Ppicos, PpicosE and Hpicos populations sampled at T_0 and T_{24} in all dilutions to determine grazing and growth rates, while ANF, HNF and ciliate populations were only sampled in undiluted water at T_0 and T_{24} to determine growth rates.

Chl *a* was determined fluorometrically by filtering 250 to 500 ml of sample onto GF/F Whatman glass fibre filters. The filters were then ground and chl *a* extracted in 90% acetone following the methods of Strickland & Parsons (1972) using a Perkin-Elmer fluorometer. Samples for size fractionation were prefiltered through a 22 µm nylon screen or 5 µm polycarbonate filters, with care being taken not to overload the latter. Chl *a* was calculated for >22, 5 to 22 and <5 µm size fractions based on difference.

Microzooplankton samples were preserved in 10% Lugol's iodine before being counted at 100 to 400× magnification on a Lietz Diavert inverted microscope. The observed ciliate taxa were separated into tintinnids, large oligotrichs (i.e. *Strombilidium* sp. and *Strombidium* sp.) and small oligotrichs (predominantly, *Lohmanniella* sp., *Leegardiella* sp. and *Halteria* sp.). Identifications were based primarily on Kofoed & 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 3 groups calculated. The unfilled portions of the tintinnids' loricae were ignored when determining biovolumes.

Samples collected for HNF and ANF enumeration were passed through a 22 µm nylon mesh and fixed with an identical volume of ice-cold glutaraldehyde (2% final concentration) for 1 h (Sanders et al. 1989). Fixed samples were filtered onto pre-stained 0.8 µm black Nuclepore filters, stained for 5 min with 2 ml primulin, rinsed with 2 ml Tris HCl and then mounted on slides for enumeration using epifluorescence microscopy (Bloem et al. 1986). Between 20 and 40 randomly selected fields of view were counted per filter. The volumes filtered for counts were based on estimates of the final cell concentration and varied with both the time of year and level of dilution.

Samples (20 ml) for bacterial enumeration were fixed with 1 ml of formaldehyde and stained with acridine orange for 5 min, before being filtered onto pre-stained 0.2 µm Nuclepore filters for enumeration using epifluorescence microscopy (Hobbie et al. 1977). Samples for <5 µm 'picophytoplankton' (Ppicos + PpicoE) enumeration were passed through a 5 µm Nuclepore pre-filter before being fixed with 1 ml of paraformaldehyde (0.2% final concentration) for at least 1 h prior to filtration onto 0.2 µm Nuclepore filters. Filters were mounted in oil on slides and frozen. Enumeration using epifluorescence microscopy was conducted within 48 h to avoid the fading of fluorescent pigments (Hall 1991).

Cell carbon for Hpicos was estimated using 11 fg C cell⁻¹ (Garrison et al. 2000). The Ppicos cells were predominately *Synechococcus* and were given a value of 235 fg C cell⁻¹ (Garrison et al. 2000). Cell carbon for PpicosE, ANF (<5 µm) and HNF (5 to 22 µm) was estimated using the organic carbon content of cells calculated from cell volumes using the formulae representing geometric solids that approximate shape (Rott 1981) and the regression equation of Eppley et al. (1970). Phytoplankton biomass measured as chl *a* in the 5 to 22 µm size class was converted to carbon using a carbon:chl *a* ratio of 30 as reported for the phytoplankton at this site by Gallegos & Vant (1996).

Microzooplankton net growth rate was calculated from changes in the biovolume of ciliates and HNF in the undiluted treatments. GMPD adjustments were made using either the mean ciliate growth rate or the mean HNF growth rate. Adjustments to grazing loss rates on chl *a* (<200 and <22 µm), ANF and HNF were made using the ciliate growth rate estimated during the Total grazing experiment. GDMP adjustments to grazing loss rates on chl *a* (<5 µm), PpicosE, Ppicos and Hpicos were made using nanoflagellate growth rate estimated during Nano grazing experiments. An example of a linear and non-linear growth response curve is given in Fig. 2. Non-linear growth response curves indicative of non-linear feeding kinetics occurred 23% of the time across all experiments, occurring least often in both Hpicos and HNF, Total

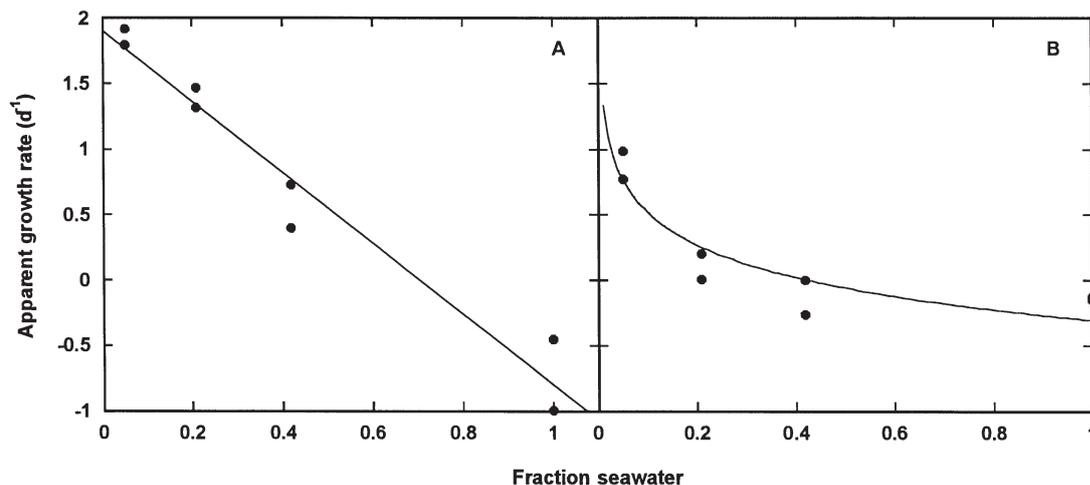


Fig. 2. Dilution experiments at Manukau Harbour. (A) An example of linear grazing results for prokaryotic picophytoplankton in January 1997. (B) An example of a non-linear growth response curve indicative of non-linear feeding kinetics for prokaryotic picophytoplankton in July 1997

grazing experiments (10%), and most often in Ppicos, Nano grazing experiment results (37%). The corrected phytoplankton grazing and growth rates were between 37–81% and 30–78% higher, respectively, than the linear rates.

To identify major seasonal differences, the growth and grazing results for all biological groups were split into 4 seasons spring (September to November) summer (December to February), autumn (March to May) and winter (June to August).

RESULTS

Phytoplankton (autotrophic cell) abundance

No late summer bloom occurred during our experiments. Consequently, chl *a* measured in the 1996 to 1997 period ranged between 1 and 10 mg m⁻³, which is well within the range reported in the harbour but lower than results often reported in the north-east region during the late summer bloom (Gallegos et al. 1996, Vant & Safi 1996). A similar range of values occurred in the 5 to 22 and <5 μm size fractions which ranged from 0.36 to 4.6 and 0.43 to 4.0 mg m⁻³, respectively. The >22 μm size fraction, however, remained relatively low throughout the year, ranging from <0.1 to 2.6 mg m⁻³.

Ppicos numbers were relatively stable throughout the year. They ranged from 2.0 × 10³ cells ml⁻¹ in October 1996 to 12 × 10³ cells ml⁻¹ in March 1997 (Fig. 3A). PpicosE numbers were higher and more variable than Ppicos, ranging from 4.0 × 10³ cells ml⁻¹ in May to 28 × 10³ cells ml⁻¹ in September 1997 (Fig. 3A).

Total ANF (<22 μm) abundance ranged from 923 cells ml⁻¹ in July to 7631 cells ml⁻¹ in December 1996

(Fig. 3B). The population declined between December 1996 and July 1997, and increased again in August 1997. Less than 10% of ANF cell numbers in the <22 μm size class were found to be <5 μm in size (<5 μm AF).

Heterotrophic abundance

Total ciliate numbers were highest in January and consisted predominantly of large and small oligotrichs (Fig. 3C). Tintinnid numbers were variable but increased during the period February to May before declining to <300 cells l⁻¹ in August through to November 1997. Large oligotrichs numbers peaked in January and increased again in July. Small oligotrichs numbers followed a similar pattern but peaked in December 1996 and again in August 1997 (Fig. 3C).

HNF abundance ranged from a mean of 1125 cells ml⁻¹ in May to 3534 cells ml⁻¹ in December (Fig. 3B). The population declined between November 1996 and July 1997, and increased again in August 1997.

Hpicos abundance ranged from 8 × 10⁵ cells ml⁻¹ in October 1996 to 68 × 10⁵ cells ml⁻¹ in December 1996 (Fig. 3D). With the exception of October to December 1996, bacterial numbers remained relatively stable around 40 × 10⁵ cells ml⁻¹.

Composition of microzooplankton grazer populations

Ciliate and HNF numbers were also converted by size into cubic volumes (μm³) in order to compare their potential grazing impacts. Ciliates dominated in the to-

tal grazing experiments, representing on average 63% of the microzooplankton biomass. HNF represented on average 33% of the total microzooplankton biomass.

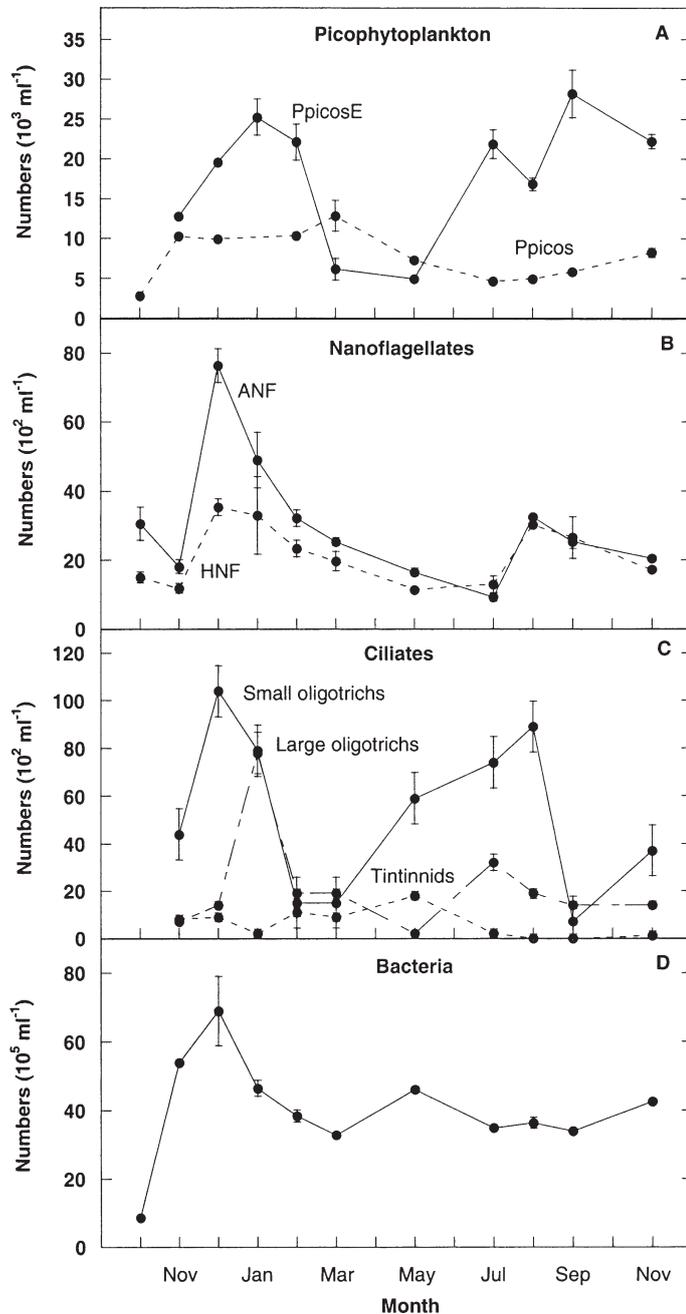


Fig. 3. Seasonal variation in microbial food web organisms between October 1996 and November 1997. (A) Eukaryotic picophytoplankton (<5 μm , PpicosE, —) and prokaryotic picophytoplankton (<5 μm , Ppicos, - - -) numbers. (B) Autotrophic nanoflagellate (ANF, —) and heterotrophic nanoflagellate (HNF, - - -) numbers. (C) Ciliate numbers by group, small oligotrichs (—), large oligotrichs (- - -) and tintinnids (· · · ·). (D) Bacterial numbers. Error bars show \pm SE

In the Nano experiments, most of the ciliates were removed by size fractionation at 22 μm . The remaining ciliates were found to represent only 1 to 5% of the microzooplankton biomass. The only exception was during May when small ciliates represented a maximum of 20% of the total microzooplankton biomass. HNF dominated the Nano experiments, representing on average 92% of the observed microzooplankton population.

In addition the purely autotrophic ciliate species *Mesodinium rubrum* was also observed occasionally in the <22 μm ciliate population. The occurrence of this species in the <22 μm ciliate population means that the grazing impact by ciliates in the Nano experiments was overestimated because *M. rubrum* does not graze but was still included in the ciliate biomass.

Grazing on phytoplankton (autotrophic) prey

Grazing by microzooplankton on phytoplankton biomass (chl *a*) in the Total grazing experiment accounted for on average 67 and 37% of the production over the year, % production grazed = $100 \times [1 - \exp(-g)]/[1 - \exp(-\mu)]$ (where g = grazing and μ growth), in the <5 and 5 to 22 μm size classes, respectively, with no measurable grazing being observed in the 22 to 200 μm size fraction.

Grazing on and growth of the ANF population was assessed only in the Total grazing experiments. ANF growth varied between 0.5 d^{-1} in December and 1.6 d^{-1} in July with the seasonal trends indicating higher growth rates during the winter and spring months (Fig. 4A). ANF populations were grazed most heavily in autumn-winter and least in spring-summer when the peak of the <5 μm phytoplankton occurred. Grazing loss rates on ANF varied between 0.1 d^{-1} in February and 1.1 d^{-1} in July (Fig. 4B). The growth (μ):grazing (g) ratio in ANF varied between 0.9 and 2.0, except for January when the ratio was 4.0.

Grazing on PpicosE populations was measured both in the Total and Nano experiments. The overall results indicated that there was no significant difference between the $\mu:g$ ratio in the 2 experiments (paired t -test: $t = 1.48$, $p = 0.09$) and a similar result was also found when we analysed growth and grazing results independently. However, seasonal analysis revealed that during autumn, the Total grazing experiments had a significantly higher rate of grazing at the 0.05 level (paired t -test: $t = 7.19$, $p = 0.04$) and a significantly higher $\mu:g$ ratio at the 0.05 level (paired t -test: $t = 7.20$, $p = 0.04$). This indicated that the addition of >22 ciliates at this time led to increased grazing. Given the overall similarity of the results (as would be expected), growth and grazing loss rates in both the Total and Nano grazing experiments followed similar trends throughout the year. In the Nano experiments, PpicosE

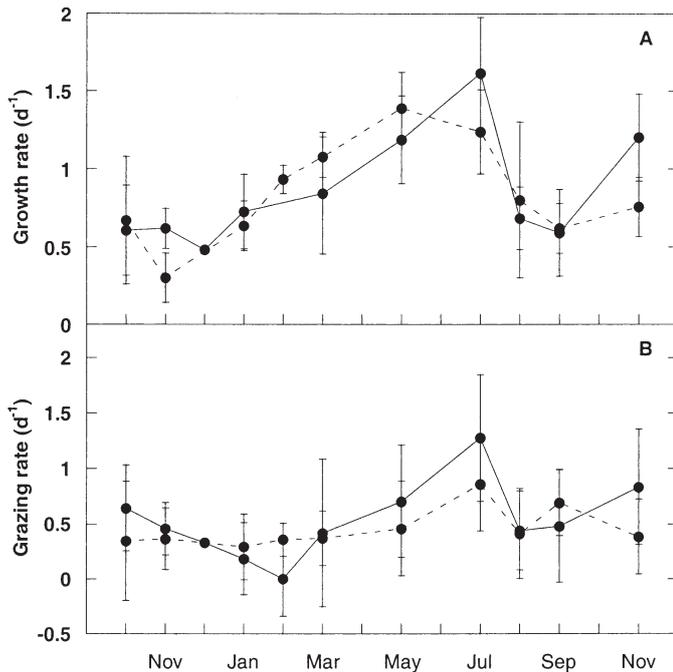


Fig. 4. (A) Seasonal variation in growth rates of nanoflagellates from Total experiments only. (B) Seasonal variation in microzooplankton grazing rates on nanoflagellates. (—) ANF, (---) HNF. Error bars show 95% confidence limits calculated from linear regressions

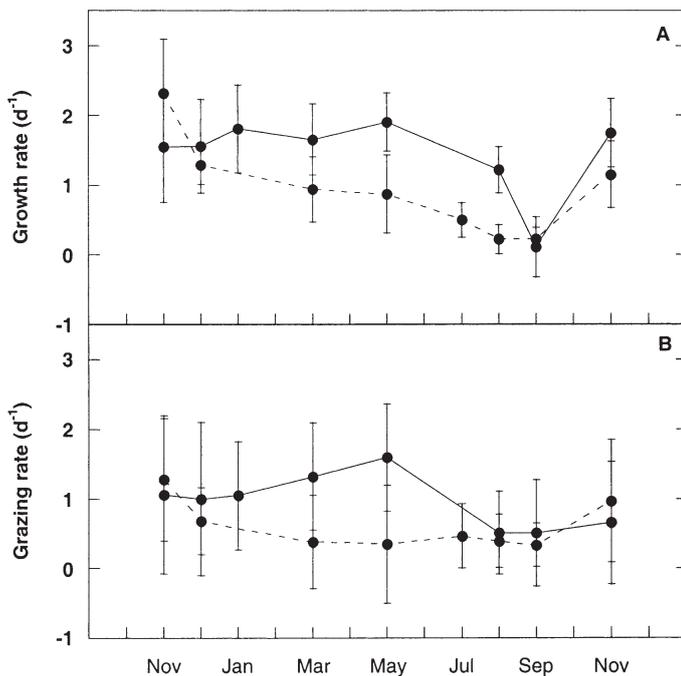


Fig. 5. (A) Seasonal variation in growth rates of eukaryotic picophytoplankton (PpicosE). (B). Seasonal variation in microzooplankton grazing rates on PpicosE. (—) Total grazing experiments, (---) Nano grazing experiments. Error bars show 95% confidence limits calculated from linear regressions

growth was on average higher in the spring and summer months, ranging between $0.2 d^{-1}$ in August and September 1997 to $2.3 d^{-1}$ in November 1996 (Fig. 5A). PpicosE grazing loss rates in the Nano experiments were on average $0.4 d^{-1}$ in March to September 1997, with a maximum $1.3 d^{-1}$ in November 1996 (Fig. 5B).

Grazing on Ppicos populations was also measured in the Total and Nano experiments. The results overall indicated that there was no significant difference between the results of the $\mu:g$ ratio in the 2 experiments (paired t -test: $t = 0.08$, $p = 0.46$). Seasonal analysis again revealed that during autumn, the Total grazing experiments had a significantly higher rate of grazing at the 0.05 level (paired t -test: $t = 7.04$, $p = 0.04$). This result, however, reflected a change in growth rates and was not found to be significant when looking at changes in the $\mu:g$ ratio at the 0.05 level (paired t -test: $t = 3.16$, $p = 0.08$). Seasonal trends in growth and grazing loss rates in both the Total and Nano grazing experiments were again similar.

Ppicos population's growth rates measured in the Nano experiments ranged between $0.2 d^{-1}$ in December and $1.6 d^{-1}$ in June (Fig. 6A). Grazing loss rates in the Nano grazing experiments varied between $0.4 d^{-1}$ in March 1997 and $1.4 d^{-1}$ in November 1996, with no clear seasonal pattern observed (Fig. 6B). The ratio of growth to grazing showed no seasonal trend, with grazing and growth rates balanced throughout the year indicating strong grazer pressure (Fig. 6).

Grazing on heterotrophic prey

Grazing on the Hpicos (bacteria) population was measured in both the Total and Nano experiments. The results again indicated that there was no significant difference in the $\mu:g$ ratio between the results of the 2 experiments (paired t -test: $t = 0.86$, $p = 0.21$). This suggests that in both experiments, HNF were the primary grazers. Hpicos growth rates measured in the Nano grazing experiments range from $2.0 d^{-1}$ in November 1996 to $0.9 d^{-1}$ in May 1997 (Fig. 7A). Growth was generally higher in the spring and declined in the winter months. Grazing loss rates varied between $2.7 d^{-1}$ in December 1996 and $0.6 d^{-1}$ in May 1997 (Fig. 7B). The ratio of growth to grazing showed no seasonal pattern, and ranged between 0.6 and 1.8.

HNF growth in the Total grazing experiments varied between $0.3 d^{-1}$ in November 1996 and $1.4 d^{-1}$ in May 1997, with the seasonal pattern indicating that the highest growth rates occur during the spring and autumn (Fig. 4A). HNF populations were most heavily grazed between May and July and least in October 1996 and March 1997, with grazing loss rates varying

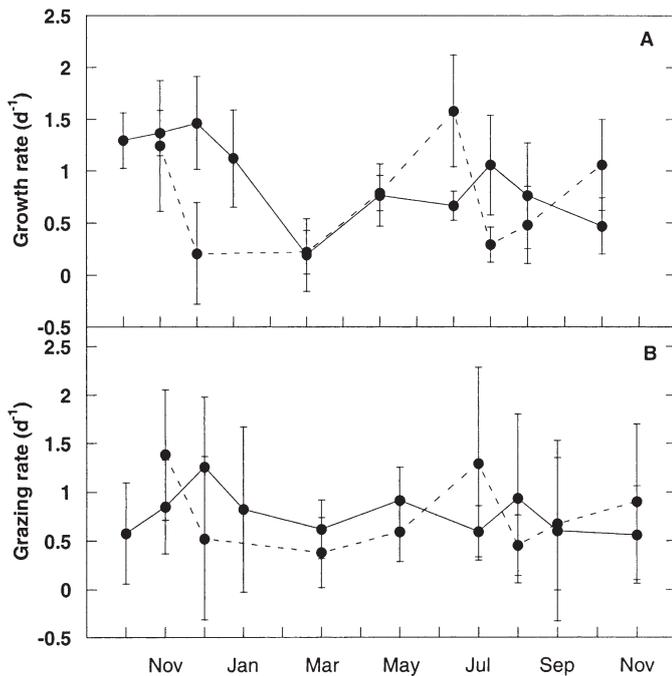


Fig. 6. (A) Seasonal variation in growth rates of prokaryotic picophytoplankton (Ppicos). (B) Seasonal variation in microzooplankton grazing rates on Ppicos. (—) Total grazing experiments, (---) Nano grazing experiments. Error bars show 95% confidence limits calculated from linear regressions

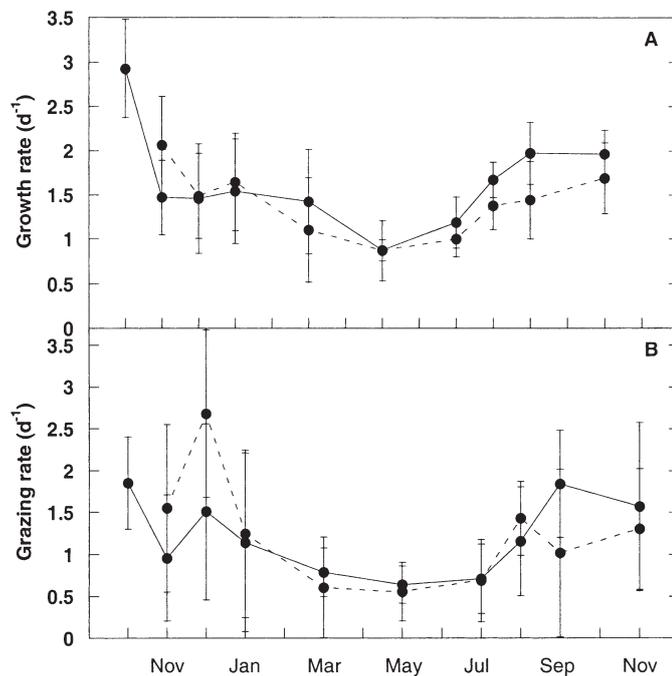


Fig. 7. (A) Seasonal variation in growth rates of Heterotrophic prey (Hpicos). (B) Seasonal variation in microzooplankton grazing rates on Hpicos. (—) Total grazing experiments, (---) Nano grazing experiments. Error bars show 95% confidence limits calculated from linear regressions

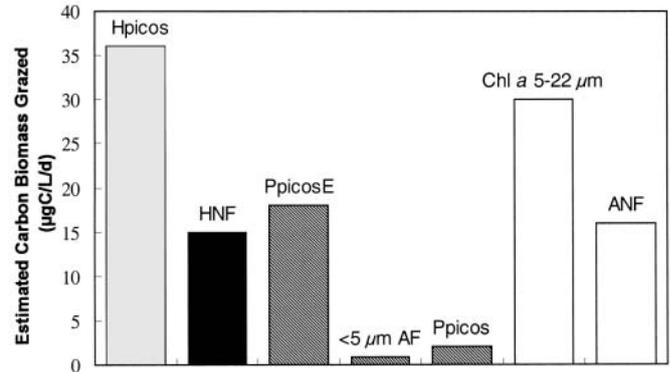


Fig. 8. Estimated mean carbon biomass grazed in each of the prey categories investigated in the study. (□) Heterotrophic prey <5 µm (Hpicos), (■) heterotrophic prey 5 to 22 µm (HNF), (▨) autotrophic prey <5 µm (PpicosE, <5 µm AF, Ppicos) and (□) autotrophic prey 5 to 22 µm (chl a 5 to 22 µm, ANF)

between 0.3 d^{-1} in January and 0.7 d^{-1} in July (Fig. 4B). The $\mu:g$ ratio in HNF varied between 0.8 and 3.0 d^{-1} , with high values being recorded between December 1996 and May 1997.

Phytoplankton (autotrophic) carbon versus heterotrophic carbon

To compare the importance of heterotrophic and autotrophic prey in the <5 µm size class, we converted the biomass of all prey organisms in the <5 µm size class into carbon, then multiplied these figures by the corresponding HNF grazing loss rates reported for each of these organisms (Fig. 8). No grazing rate could be determined for the <5 µm AF; therefore, to estimate carbon grazed in this group, we used the grazing rate of HNF on the PpicosE component. Hpicos prey represented on average 1.9 times the carbon biomass of the <5 µm phytoplankton. We also estimated the relative importance of heterotrophic and autotrophic prey in the 5 to 22 µm size class. Again, we converted the biomass of all prey organisms in the 5 to 22 µm size class into carbon then multiplied these figures by the corresponding ciliate grazing loss rates reported for each of these organisms. HNF prey represented on average 32% of the carbon grazed in the total grazing experiment (Fig. 8).

DISCUSSION

Grazing on autotrophic components of the food web

The total microzooplankton biomass in Manukau Harbour was dominated by large ciliates (>22 µm),

with HNF dominating the nanoplankton (<22 μm) sized grazers and also being a major contributor to the total biomass. This situation is in agreement with other studies in both open ocean and coastal waters, reporting ciliates as the dominant microzooplankton (Capriulo & Carpenter 1980, Epstein & Shiaris 1992). In this study, microzooplankton populations were capable of removing large amounts of the production of chl *a* in the <5 and 5 to 22 μm size classes (67 and 37 %, respectively). These results were within the ranges reported by Gallegos et al. (1996) for Manukau Harbour and reported elsewhere for microzooplankton grazing on chl *a* (Verity & Vernet 1992, Froneman & Perissinotto 1996). Gallegos et al. (1996) found microzooplankton grazing removed on average 90 and 20% of the production of chl *a* in the <5 and 5 to 22 μm size classes, respectively, with no measurable grazing on the 22 to 200 μm size class. The results of this study confirm that microzooplankton are the main consumers of the <5 μm phytoplankton biomass in Manukau Harbour, and also have a significant impact on the larger (5 to 22 μm) phytoplankton at times.

The inclusion of separate grazing loss rates calculated specifically for microzooplankton grazing on ANF populations also allows us to at least partially separate out which components of the 5 to 22 μm chl *a* size fraction were preferentially grazed. The results showed an average of 72 % of ANF production was grazed compared to 37 % of chl *a* production in the 5 to 22 μm size fraction. This indicates that microzooplankton had a preference for ANF over other, predominantly diatom, photosynthetic prey in the 5 to 22 μm size class. This is consistent with Burkill et al. (1987), who showed that microzooplankton have a preference for dinoflagellates, cryptophytes, chlorophytes and prasinophytes over diatoms. The results indicate that when ANF represent a large part of the 5 to 22 μm size class, grazing by microzooplankton may enhance the transfer of carbon to higher trophic levels within the food web at this site.

The more heavily grazed <5 μm phytoplankton population was broken down into 3 major components in this study: the PpicosE, Ppicos and <5 μm ANF. These represented 45 to 83, 2 to 41 and 2 to 10% of the observed biomass in the <5 μm phytoplankton size class, respectively, during the year. The ratio of these organisms within the <5 μm size class is within the range reported in other estuarine environments (Vaqué et al. 1992).

Grazing on the <5 μm AF components could not be determined due to the low and inconsistent number reported in this group; however, this group was only a small contributor to the <5 μm biomass (2 to 10%). Grazing was measured specifically on PpicosE, with the Total grazing experiments results showing on aver-

age 89 % of the production of PpicosE was grazed. Due to the removal of larger ciliates, the Nano grazing experiment showed lower grazing pressure, with an average of 75 % of the production being grazed. Grazing experiments indicated that Ppicos were preferentially grazed. The Total grazing experiment results showed on average 90 % of weight-specific production of Ppicos was grazed, while the Nano grazing experiment showed on average over ~100 % (1.03) of the weight-specific production of this group was grazed. These results indicate heavy microzooplankton grazing on all the <5 μm phytoplankton, but preferential grazing on the smaller Ppicos dominated by the *Synechococcus*, and show the importance of establishing the contribution of different components to the microbial food web. The ratio of different organisms is likely to impact significantly on the rate of transfer of carbon to higher trophic levels within the food web.

As indicated previously, the design of the Nano grazing experiments led to the exclusion of most ciliates, meaning that HNF dominated in these experiments. The results of paired *t*-tests between the 2 experiments indicated that the exclusion of ciliates made no significant difference to the grazing loss rates on PpicosE or Ppicos. Because of this, we can conclude that HNF were responsible for most of the grazing on these populations. Seasonal analysis of the results did, however, indicate that during autumn, the inclusion of ciliates led to significantly higher grazing on PpicosE. Although not statistically significant, a similar result was found for grazing on Ppicos. These results correspond to a period when most ciliate populations were low but small oligotrich ciliates were at their highest numbers. During May, the largest number of small ciliates was also reported in the <22 μm size class (20%). These results suggest that at this time, small ciliates around 15 to 30 μm in size contributed significantly to grazing loss rates on the <5 μm size class. This result is consistent with previous studies (Epstein & Shiaris 1992, Gallegos et al. 1996, Tamigneaux et al. 1997) that report both HNF and ciliates as significant grazers of small eukaryotes. The results overall suggest that HNF and, to a lesser extent small ciliates, play an important role by repackaging the smaller phytoplankton biomass into a size that is more accessible for larger predators and, hence, form an important link in the food web.

Grazing on heterotrophic components of the food web

Gallegos et al. (1996) suggested that heterotrophic organisms were also a potential food source for microzooplankton in Manukau Harbour. The present study shows that heterotrophic prey was an important source

of food for microzooplankton populations in the north-east corner of Manukau Harbour, with bacterial biomass at this site being 2 to 4 times higher on average than those reported in open marine waters around New Zealand (Safi & Hall 1997, Smith & Hall 1997). However, given the high organic nutrients and allochthonous organic matter reported at this site, bacterial numbers were still substantially lower than those reported in some eutrophic estuarine systems (Neilson & Cronin 1981, Coffin & Sharp 1987, Painchaud & Therriault 1989, Vaqué et al. 1992). Both the growth rates of bacteria and the grazing rates on bacteria were high during this study compared to those reported in open marine waters around New Zealand (Safi & Hall 1997, Smith & Hall 1997), indicating that bacteria were both growing rapidly and being grazed rapidly by microzooplankton. This fast turnover of the bacterial population would also help explain the elevated HNF numbers observed at this site, which were 2 to 7 times higher than those observed in marine waters around New Zealand (Safi & Hall 1997).

As there was no significant difference between the 2 types of experiments and because HNF grazers could account for 99% of the grazing reported on bacteria in the present study, it was assumed that all the microzooplankton grazing of HpicoS was by HNF. This assumption is consistent with studies in both estuarine and marine environments, which report HNF as the primary consumers of bacteria (Anderson & Fenchel 1985, McManus & Fuhrman 1986).

The HNF population is also a prey item for microzooplankton. By measuring grazing on HNF, we were able to assess this group's importance both as a direct source of carbon as prey for larger predators, and as a

key converter of smaller autotrophic and heterotrophic carbon into a form available to larger predators. Lynn et al. (1991) reported microciliates as the dominant grazers of nanoplankton, while Weisse (1991) reported that HNF were important especially for ciliate filter-feeders such as *Strobilidium* sp. Epstein et al. (1992) also found HNF constituted 17% of the entire ciliate food spectrum found in a temperate zone sandy tidal flat. Because other microzooplankton were rare and zooplankton are excluded from our experiments, we conclude that ciliates must also have dominated the grazing of HNF in our experiments.

Total microzooplankton grazing removed 63% HNF production d^{-1} . This was again a significantly higher proportion than the 37% production d^{-1} removed in the 5 to 22 μm chl a size class, but lower than the 72% reported for ANF. This shows that microzooplankton in the Total grazing experiments also appear to have a preference for HNF over photosynthetic diatom prey in this size class. This higher grazing rate also suggests that a significant part of the HNF biomass is being consumed by ciliate grazers and, hence, will help sustain the higher number of ciliates observed at this site compared to other parts of the harbour. Gallegos et al. (1996) reported ciliates at this site in 1994 were ~60% higher in numbers and biovolume than at a site in the south-east of the harbour, and up to 97% higher for small oligotrichs at this site than the harbour entrance. The grazing impacts suggest that HNF play a key role in the food web and show that at times the HNF are an important link between small heterotrophic prey and larger ciliate grazers. Although not investigated in this study, grazing on viruses may also provide an important additional heterotrophic food source as both HNF

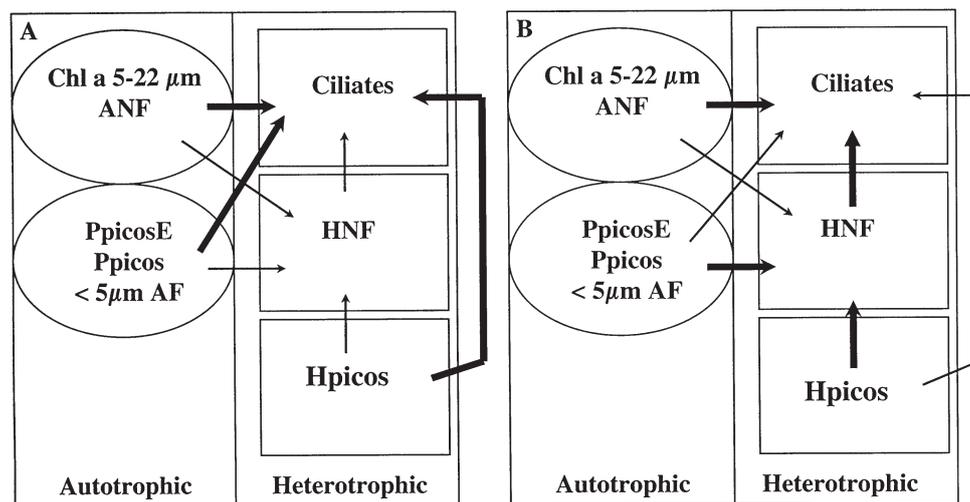


Fig. 9. Flow of biomass through the components of the microbial food web. Thin arrow indicates weak flow, thick arrow indicates strong flow. (A) The flow of biomass proposed by Gallegos et al. (1996). (B) The flow of biomass observed in this study

and ciliates have been shown to graze on viruses (Gonzalez & Suttle 1993, Azam 1998).

Importance of phytoplankton (autotrophs) versus heterotrophs as prey

Having established the major prey and predator groups and the relationships between the two, it is important to put these elements of the food web into some context of biomass, and examine the importance of each of these groups and their ability to transfer biomass through the food web. Although a significant proportion of the <5 µm phytoplankton biomass was grazed by HNF, when we examined the importance of different groups in terms of carbon, we found that grazed bacterial prey represented on average 1.9 times as much carbon as the total of the other <5 µm components. It is not uncommon for bacterial carbon to exceed phytoplankton carbon in marine environments (Li et al. 1992); however, our results show a very high growth rate and turnover in this group. Bacterial activity is probably associated with the very high levels of organic nutrients and allochthonous organic matter reported at this site, resulting from the output of the sewage treatment plant. The results also show that HNF, not small ciliates, control the bacterial biomass in this system. In turn, the results of grazing on HNF suggest that the ciliate population is at least partly sustained by grazing on the elevated HNF populations. HNF prey in the 5 to 22 µm size class represented on average 32% of the carbon grazed during the Total grazing experiment. This result is substantially higher than that reported by Epstein et al. (1992), who found that HNF constituted 17% of the entire ciliate food spectrum found in a sandy temperate zone tidal flat and appears to reflect the higher HNF biomass reported in Manukau Harbour. These results differ from those predicted by Gallegos et al. (1996), but may be indicative of other densely populated coastal areas where strong anthropogenic influences affect food web processes.

Both HNF and small ciliates are reported as grazers of bacteria in marine environments. Anderson & Fenchel (1985) and McManus & Fuhman (1986) reported HNF as the primary consumers of bacteria, while Epstein & Shiaris (1992) and Tamigneaux et al. (1997) report ciliates <20 µm as the dominant grazers of prey <5 µm. Gallegos et al. (1996) suggested that elevated levels of bacterial production may selectively stimulate the growth of small (bacterivorous) ciliates. The current study clearly shows that heterotrophic food sources are significant and at least partly sustain the microzooplankton population, but the transfer of heterotrophic biomass through the food web is less

direct than proposed by Gallegos et al. (1996) (see Fig. 9). This study has shown that HNF can play an important intermediary role between small prey such as bacteria and picophytoplankton, and the larger microzooplankton grazers such as ciliates.

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