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The importance of zooplankton-protozoan trophic couplings in Lake Michigan

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Abstract

The importance of the zooplankton-protozoan trophic coupling was determined experimentally by measured changes in protozoan growth rates with increasing zooplankton biomass. In five of six experiments conducted in Lake Michigan, a significant inverse relationship between protozoan growth and zooplankton biomass was observed (avg r^2 = 70%). Zooplankton clearance rates on protozoan assemblages [range, 1.0–6.2 ml (μg dry wt)$^{-1}$ d^{-1}] were comparable to those previously measured for phytoplankton which suggested that protozoa are important prey for zooplankton. Clearance rates on individual protozoan taxa [0–15.6 ml (μg dry wt)$^{-1}$ d^{-1}] were size-dependent. Rates were greatest for taxa <20 μm in size (mainly nanoflagellates and small ciliates). In contrast to findings for phytoplankton, no evidence emerged for grazer resistance nor growth enhancement by planktonic protozoa in response to grazing. The high flux rates for macrozooplankton on heterotrophic nanoflagellates observed in all experiments (0.2–6.0 μg C liter^{-1} d^{-1}) provided evidence that a large fraction of picoplankton C may be directly transferred to higher trophic levels via a picoplankton-flagellate-zooplankton coupling.

Debate continues as to whether macrozooplankton (rotifers, crustacean zooplankton) can effectively graze on components of the microbial food web (picoplankton, protozoa), and thus, whether a significant amount of C is transferred from picoplankton to higher trophic levels (e.g. Sherr et al. 1987). Recent studies suggest that picoplankton-sized cells can be ingested by macrozooplankton; however, picoplankton production is not efficiently grazed or grazing may be intermittent (Pace et al. 1990). Moreover, the harvesting efficiency of macrozooplankton on picoplankton cells varies among age classes and species of zooplankton consumers (Pace et al. 1983). Because most grazing on picoplankton is by protozoa (Sanders et al. 1989; Fahnenstiel et al. 1991), without information on the fate of protozoa (Weisse et al. 1990) it remains difficult to assess whether picoplankton production is an important trophic link to macrozooplankton.

Information is limited on macrozooplankton predation on protozoa (*see* Stoecker and Capuzzo 1990). Of the studies that do exist, most are laboratory investigations which have demonstrated that single copepod species can actively graze protozoa and that a diet composed entirely of flagellated or ciliated protozoa can support growth and reproduction of the macrozooplankton species tested (e.g. Stoecker and Egloff 1987; Sanders and Porter 1990). There is also some indication that copepods prefer protozoa over algae and that macrozooplankton taxa fed seston ingest particles in the nano- to microplankton size range (e.g. Nival and Nival 1976). Little is known, however, about predation by intact zoo-

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This paper is dedicated to the memory of Hunter Joseph Carrick, Sr.

plankton assemblages on naturally occurring planktonic protozoa (Kleppel et al. 1988). Laboratory findings cannot be accurately extrapolated to the field because grazing is not equal among co-occurring macrozooplankton species (e.g. Paffenhöfer and Knowles 1980). Moreover, although grazer resistance is a common adaptation of phytoplankton (Porter 1976; Lehman and Sandgren 1985), the ability of protozoa to withstand or avoid predation is largely unknown.

The specific objectives of this study were to determine macrozooplankton clearance rates on protozoa, assess whether macrozooplankton feed selectively on protozoa of a particular size, and evaluate the quantitative importance of carbon flux from protozoa to macrozooplankton. We present results based on six experiments conducted in 1989 (March–October) in Lake Michigan. These experiments are unique, in that grazing was measured on natural assemblages of protozoa by a complete macrozooplankton community while the concentration and composition of both plankton components were maintained at levels representative of ambient lake conditions. We believe the results of this investigation are probably representative of many unproductive freshwater and marine systems because Lake Michigan is a large (surface area, 58,000 km^2; mean depth, 84 m), oligotrophic lake with chlorophyll concentrations ranging from 0.3 to 3.0 μg $liter^{-1}$ and total P values varying between 4 and 8 μg $liter^{-1}$ (Schelske et al. 1986). Moreover, the composition and abundance of picoplankton (Scavia and Laird 1987; Fahnenstiel and Carrick 1991) and protozoa (Carrick and Fahnenstiel 1989, 1990) are similar to those in various freshwater and marine ecosystems.

Methods

Ambient conditions—Sampling was conducted at a single offshore station in Lake Michigan (43°1′11″N, 86°36′48″W; max depth, 100 m) on seven occasions in 1989 (29 March, 19 April, 10 May, 13 June, 10 July, 28 August, and 4 October). For all analyses, water was collected from the surface mixed layer (5 m) with a 5-liter or 30-liter PVC Niskin bottle at dusk (2000–2200 hours). Water-column temperature profiles were measured with an electronic bathythermograph. Ambient protozoan abundances were determined from lake-water samples transferred into 250-ml amber bottles and preserved with either 1% Lugol's acid iodine (ciliate and microflagellate samples) or 1% glutaraldehyde buffered with 0.1 M sodium cacodylate (nanoflagellate samples). Because of the large range in both cell size and abundance among protozoa, the abundance of microprotozoa (microflagellates and Ciliophora, most >20 and <200 μm in size) and nanoprotozoa (nanoflagellates <20 μm in size) was measured separately. Moreover, potential trophic status of nanoprotozoa was assessed by the presence (phototrophic, Pnano) or absence (heterotrophic, Hnano) of pigmentation (*see below*).

Nanoprotozoa were enumerated with epifluorescence microscopy from slides prepared within 24 h of sampling. Subsamples (10–20 ml) were filtered onto prestained (Irgalan Black) 0.8-μm pore-size Nuclepore filters that were subsequently stained with primulin. Filters were mounted between a microscope slide and coverslip with immersion oil (Caron 1983). Microprotozoan biomass and community composition were determined with the Utermohl technique whereby subsamples (25–50 ml) were settled onto coverslips and systematically enumerated with an inverted microscope (400×). Cellular volume estimates for Pnano and microflagellates were converted to C based on Strathmann (1967) conversion factors; Hnano and ciliate cell volumes were converted to C with the conversion factor (cell volume × 0.15 g C ml^{-1}) of Laws et al. (1984). C estimates were corrected for cell shrinkage due to preservation (Choi and Stoecker 1989). Protozoan systematics used here conform to those presented by Lee et al. (1985).

Macrozooplankton abundance was determined from vertical net hauls (0.5-m aperture, 153-μm mesh, Wisconsin-type net) through the photic zone (0–40 m). Collected samples were then narcotized and preserved with sugar Formalin. Macrozooplankton biomass was determined by enumerating subsamples and subsequently converting

abundances to dry weight biomass with taxon-specific conversion values for Lake Michigan zooplankton (Hawkins and Evans 1979).

Zooplankton grazing experiments—The impact of macrozooplankton (organisms >153 μm in size) grazing on protozoa in Lake Michigan surface waters was determined on six of the seven sampling dates (excluding 10 May) by experimentally manipulating macrozooplankton concentrations across a series of bottles and evaluating changes in protozoan densities within the bottles over time (Lehman 1980). Collected lake water (150 liters) was screened through a 153-μm mesh-size Wisconsin-type zooplankton net and dispensed into a shaded 200-liter polyethylene tank, after which an additional 90 liters of screened lake water was dispensed into a second 200-liter tank. Epilimnetic macrozooplankton were collected with a 10-m vertical haul via a solid bucket (to avoid excessive mechanical damage to the zooplankton) and carefully added to the 90-liter tank by submerging the bucket into the collected lake water and allowing the macrozooplankton to escape.

Macrozooplankton treatments were administered by filling 10-liter carboys with screened lake water (from the 150-liter sample) and subsequently inoculating them with subsamples from the 90-liter zooplankton sample, so that concentrations in the carboys were ~1×, 1.5×, and 3× ambient macrozooplankton concentrations. Some bottles had no macrozooplankton added and served as the 0× treatment. In experiments conducted on 29 March, the 1× and 3× macrozooplankton treatments were replicated (total number of experimental containers, $n = 6$); on 19 April and 13 June, the 1×, 1.5×, and 3× treatments were replicated ($n = 7$); all four zooplankton treatments were duplicated for the remaining three experiments ($n = 8$).

To minimize the effects of nutrient recycling via zooplankton excretion to protozoa (particularly phototrophic forms), we added phosphate (0.23 μM final concn) to all bottles after thermal stratification (June through October, Scavia and Fahnenstiel 1987). We chose to add P to our bottles, because it limits phytoplanktonic growth in Lake Michigan (Schelske et al. 1986). To explore the possibility that macrozooplankton might be supplying protozoa with organic compounds which could enhance growth by either direct uptake (Haas and Webb 1979) or by augmenting bacterial prey densities (Taylor and Lean 1981), we added glucose (0.09 μM final concn) to one 0× and one 1× bottle on several dates (13 June, 10 July, 28 August, and 4 October).

All bottles were incubated for 24 h at ambient light and temperature in a shipboard incubator equipped with rotating racks. Initial and final subsamples for nano- and microprotozoa were removed from the bottles, preserved, and enumerated (as described previously) in order to estimate exponential growth by

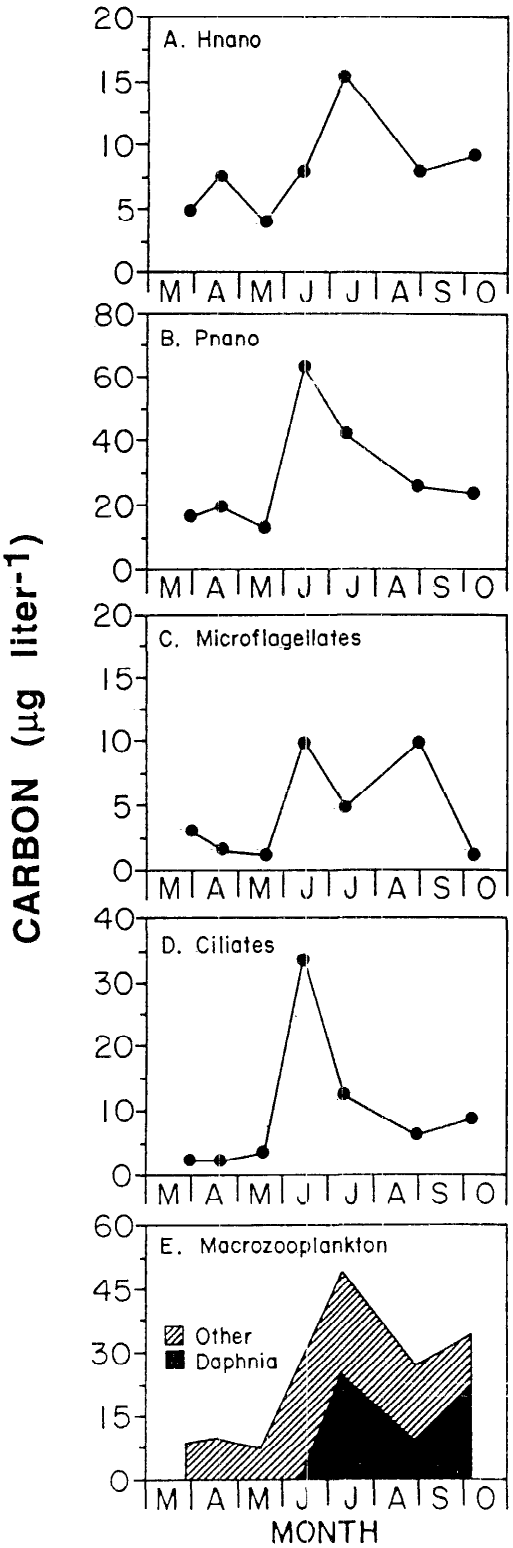
$$r = \frac{\ln(N_t/N_0)}{t}$$

where r is the rate of population growth (d^{-1}), N_0 and N_t are initial and final cell densities, and t is the duration of incubation. At the end of each experiment, macrozooplankton abundances in each bottle were determined by passing the entire contents of each carboy through a 153-μm mesh-size screen and counting the retained animals as described above.

The relationship between protozoan growth (dependent variable) and zooplankton biomass (independent variable) was assessed with simple linear regression. The slope of this relationship provides an estimate of the weight-specific zooplankton clearance rate on protozoa (μg dry wt $liter^{-1}$ d^{-1}) and the y-intercept is an estimate of the exponential growth rate (d^{-1}) of the protozoa (Lehman and Sandgren 1985). We calculated the flux of C from protozoa to macrozooplankton (μg C $liter^{-1}$ d^{-1}) by multiplying the clearance rate for the protozoan group under question by the ambient macrozooplankton biomass and, in turn, multiplying this product by the ambient biomass of the protozoan group itself.

Results

Ambient conditions—The abundance of planktonic protozoa and macrozooplankton varied temporally with biomass maximal during early to midstratification (Fig.



1). These temporal patterns seem to be typical for these planktonic components in Lake Michigan based on previous studies (Carrick and Fahnenstiel 1989, 1990; Dorazio et al. 1987).

The Hnano assemblage (Fig. 1A) was composed of colorless chrysomonads, cryptomonads, and choanoflagellates whose C concentrations ranged from 5 to 15 μg liter^{-1} throughout the course of this study and reached a maximum during midstratification (temp. >15°C, July–September). Pnano C (range, 16–63 μg liter^{-1}) was dominated by chrysomonads and cryptomonads and reached maximal concentrations during initial (temp. >4° <15°C, June) stratification (Fig. 1B). Microflagellate C was composed entirely of dinoflagellates (range, 1.2–9.8 μg liter^{-1}) and also increased during initial stratification, yet remained high until October when C mass fell to levels common for spring isothermal (temp. <4°C, March–May) assemblages (Fig. 1C). Ciliate C (Fig. 1D) was also low prior to thermal stratification (<5 μg liter^{-1}) and increased during initial stratification (33.9 μg liter^{-1}) when large spirotrichs were dominant. During midstratification, C declined to an intermediate level (5–10 μg liter^{-1}) and was composed of smaller spirotrichs and prorodontids, and this assemblage remained throughout the rest of our sampling.

Lastly, macrozooplankton C was low during the spring isothermal period and increased sharply during initial stratification (Fig. 1E). C mass was maximal during midstratification, as levels near 50 μg liter^{-1} were observed in July, and remained relatively high thereafter (~25–30 μg liter^{-1}). Macrozooplankton communities were dominated by copepods (juvenile and adult *Diaptomus* spp.) in spring, while *Daphnia* (mainly *D. galeata*) constituted a significant fraction of total zooplankton biomass after thermal stratification (avg, 51.2%).

Zooplankton grazing rates—Both nano- and microprotozoan growth rates were in-

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Fig. 1. Carbon mass among five groups of plankton sampled approximately monthly (March–October) from the surface waters of Lake Michigan in 1989.

versely related with zooplankton biomass from the bottle experiments on nearly all dates (Table 1; Figs. 2, 3). On 19 April a marginally significant regression was observed for nanoprotozoa ($P < 0.10$), while microprotozoan growth varied independently from zooplankton biomass. Zooplankton biomass accounted for 58–84% of the variation in protozoan growth and yielded estimates of zooplankton clearance rates on protozoa.

The intercept from these analyses (Figs. 2, 3) showed growth rates for nano- and microprotozoa that were lower (range, −0.17–0.47 d^{-1}) than estimates with other techniques (Carrick 1990). These conservative growth estimates are probably a result of chemical manipulations inside our experimental bottles due to the added P; however, this procedure was necessary to minimize potential differences in nutrient recycling from zooplankton to protozoan prey at differing macrozooplankton abundance (Lehman 1980). The reasonably strong relationship derived from these experiments demonstrates the validity of this assumption, despite conservative estimates of growth. Lastly, neither nano- or microprotozoa were affected by adding glucose to 0× and 1× bottles, which suggests that the growth of these organisms is not currently limited by this organic compound. This conclusion is tentative because it is difficult to ascertain growth responses to nutrient addition with such short incubation periods (Healy 1979).

The range in weight-specific clearance rates for macrozooplankton on nano- and microprotozoa varied from 1.6 to 6.2 and from 1.0 to 4.8 ml (μg dry wt)$^{-1}$ d^{-1} (Table 1). Average clearance rates on Hnano were nearly twofold higher than those on Pnano, while clearances of microflagellates and ciliates were similar (Table 2). In addition, both nanoprotozoan and microprotozoan clearance rates increased during thermal stratification and were correlated with increasing macrozooplankton biomass ($r = 0.87$, $n = 6$, $P < 0.01$; and $r = 0.83$, $n = 6$, $P < 0.01$, respectively).

Zooplankton selectivity—A great range was observed in the weight-specific clearance by macrozooplankton on abundant protozoan populations [0–15.6 ml (μg dry wt)$^{-1}$ d^{-1}] (Table 2). On four of six experiment dates, protozoan taxa in the <7-μm size range (*Chromulina* sp. 1, *Chromulina* sp. 2, *Ochromonas* sp., and *Katablepharis ovalis*) were cleared by macrozooplankton at greater rates than other protozoa, while larger (>30 μm) protozoa (*Strombidium viride, Strobilidium velox,* and *Ceratium hirudinella*) were grazed only during thermal stratification, which coincided with increased zooplankton biomass. Moreover, zooplankton clearance rates decreased with increasing cell size; the average clearance rates for 16 common protozoan prey (Table 2) were negatively correlated with cell size (Fig. 4). Variation in the average clearance rate for these 16 taxa on each date did not correlate with changes in macrozooplankton biomass or composition. Consistent with this observation, only one taxon (*Vorticella* sp.) was unaffected by increasing macrozooplankton biomass on each of the three dates that it was abundant in the water column (Table 2).

Table 1. Summary of regression analyses assessing the relationship between the growth of nano- (Hnano and Pnano) and micro- (microflagellates and ciliates) protozoa and increasing macrozooplankton biomass (μg liter^{-1} dry wt) in Lake Michigan.

1989, temp.	Protozoan group	Clearance [ml(μg dry wt)$^{-1}$ d^{-1}]	Flux (μg C liter^{-1} d^{-1})	r^2	Prob.
29 Mar, 2.0°C	Nano	2.6	0.99	68.3	0.043
	Micro	1.4	0.14	70.1	0.038
19 Apr, 2.9°C	Nano	4.5	2.43	50.0	0.076
	Micro	2.0	0.16	8.0	0.538
13 Jun, 10.0°C	Nano	2.1	8.03	84.4	0.003
	Micro	1.0	2.36	58.2	0.046
10 Jul, 19.0°C	Nano	4.2	24.20	66.9	0.013
	Micro	4.7	8.28	83.7	0.001
28 Aug, 21.5°C	Nano	6.2	11.31	74.3	0.006
	Micro	4.8	4.24	85.1	0.001
4 Oct, 13.8°C	Nano	1.6	3.57	68.6	0.011
	Micro	1.2	0.82	75.9	0.005

Discussion

Macrozooplankton grazing on protozoa—Few studies have measured macrozooplankton grazing on protozoa in the field. The present study is unique because we were

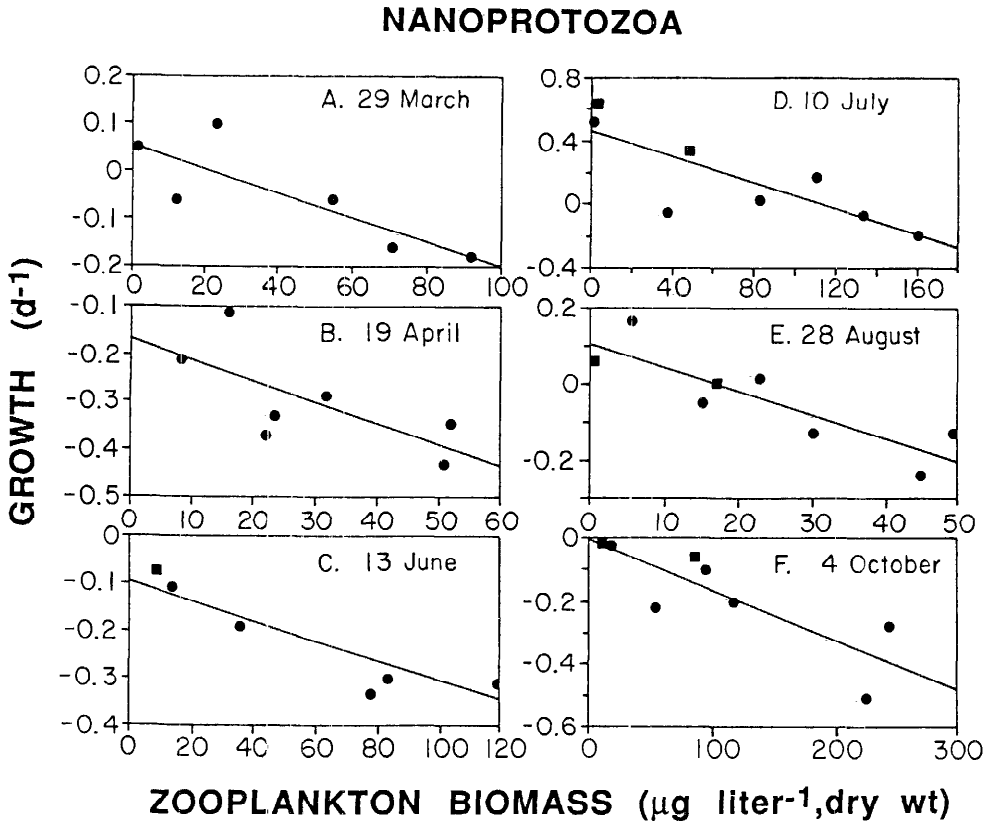

Fig. 2. Nanoprotozoan growth rates regressed onto increasing zooplankton biomass for surface communities in Lake Michigan sampled in 1989. (Bottles with 0.09 μM glucose added—■.) Regression statistics are presented in Table 1.

able to assess macrozooplankton predation on protozoa, while maintaining reasonably natural composition and concentration of both plankton components. Our results indicate that nano- and microprotozoa are consistently ingested by macrozooplankton in Lake Michigan, and that zooplankton biomass explained >70% of the variation in protozoan growth (on average and excluding the nonsignificant regressions on 19 April).

In contrast to other studies that estimated macrozooplankton grazing on components of the microbial food web (e.g. Pace et al. 1983, 1990), we found that protozoa were consistently important prey items for epilimnetic macrozooplankton in Lake Michigan. Average estimates of macrozooplankton clearance rates on nano- and microprotozoa determined from our experiments [3.53 and 2.52 ml (μg dry wt)$^{-1}$ d^{-1}] were at the high end of the range for rates reported on epilimnetic phytoplankton in Lake Michigan [range, 0–2.6 ml (μg dry wt)$^{-1}$ d^{-1}, Scavia and Fahnenstiel 1987]. Also, clearance rates of smaller Hnano cells (avg, 4.1; SD = 1.9) by macrozooplankton determined here were almost twofold higher than clearance rates measured for phytoplankton both in surface and deep regions of Lake Michigan (Scavia and Fahnenstiel 1987; Fahnenstiel and Scavia 1987).

Laboratory studies indicated that macrozooplankton have higher ingestion rates for ciliates than for algae (e.g. Stoecker and Egloff 1987). Some field studies showed that a natural assemblage of microzooplankton (ciliates and zooflagellates) comprised a large portion of the C ingested by estuarine copepods (Gifford and Dagg 1988) and that

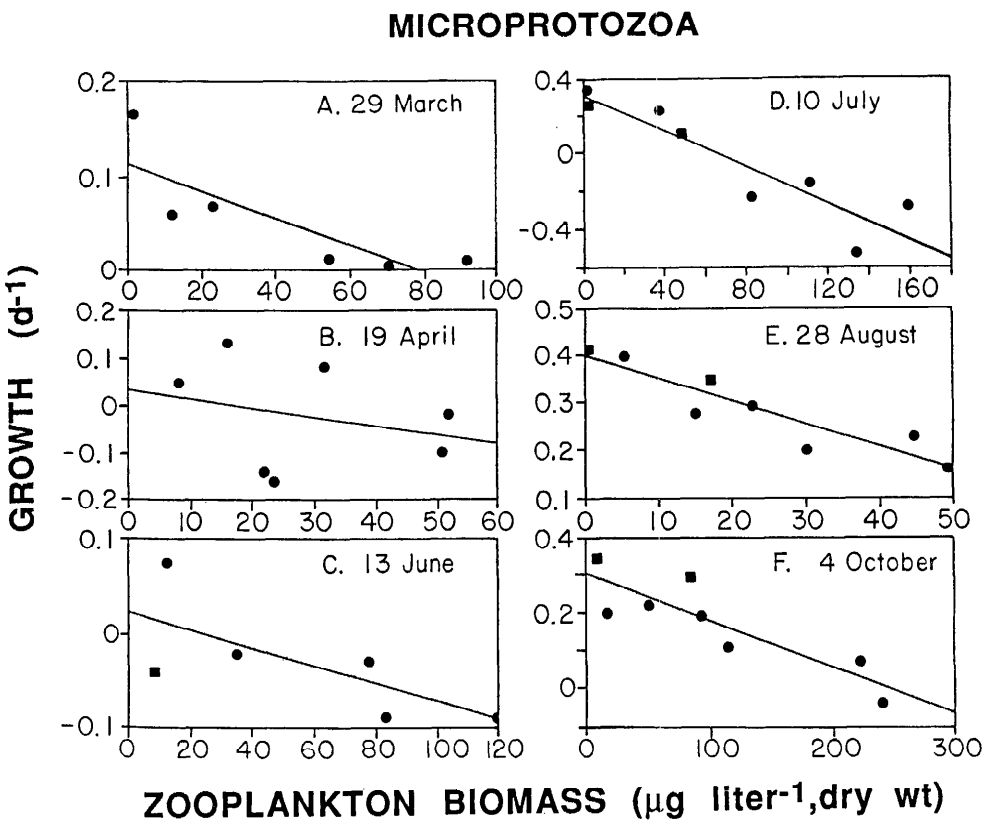

Fig. 3. As Fig. 2, but for microprotozoan growth rates.

heterotrophic flagellates could be important food for copepods, particularly at lower phytoplankton densities (Roman et al. 1988). In addition, a model of grazing control of phytoplankton in the open subarctic Pacific Ocean could be balanced only by assuming that macrozooplankton are omnivorous and graze on both phytoplankton and protozoa (Frost 1987). Although our data do not demonstrate macrozooplankton selection for protozoa over phytoplankton, they suggest that protozoa are significant prey items in addition to phytoplankton in Lake Michigan.

High macrozooplankton clearance rates on protozoa might be a function of their high food quality (Stoecker and Capuzzo 1990). Consistent with this idea, a diet of ciliates similar to some of those occurring in Lake Michigan (*Strombidium* and *Urotricha*) was shown to enhance egg production of the calanoid copepod *Acartia tonsa* (Stoecker and Egloff 1987). Also, Cladocera such as *Daphnia* were found to grow and reproduce on a diet composed entirely of heterotrophic flagellates (Sanders and Porter 1990), and comparatively, mixed nanoflagellates enhanced the growth and survivorship of *Daphnia* over other algal diets (DeBiase et al. 1990).

Selective grazing by zooplankton on protozoa—Although the average clearance rates on individual protozoan taxa showed considerable variation, rates were generally higher for taxa <20 µm in equivalent spherical diameter. Other studies assessing macrozooplankton predation on natural plankton assemblages reported similar results in that adult copepods feed most readily on cells 3–7 µm in size (Nival and Nival 1976). Moreover, freshwater plankton in the 3–30-µm size range are preferred by calanoid copepods (Vanderploeg 1981) and filter-feeding Cladocera (Gliwicz 1980). The relative

Table 2. Examples of weight-specific clearance rates [ml(μg dry wt^{-1})d^{-1}] of macrozooplankton on 16 common protozoa from the surface waters of Lake Michigan in 1989.

Taxon	ESD*	29 Mar	19 Apr	13 Jun	10 Jul	28 Aug	4 Oct	Mean
Hnano								
Chromulina sp. 1	2.5	3.3	10.3	3.2	4.2	8.0	2.1	5.2
Chromulina sp. 2	2.7	5.7	—	5.1	—	3.9	—	4.9
Ochromonas sp.	2.7	—	0.1	4.1	3.2	15.6	1.0	4.8
Katablepharis ovalis	6.2	—	—	0	3.4	3.8	3.4	2.7
Pnano								
Chrysochromulina parva	3.7	—	0	1.8	4.2	11.8	1.9	3.9
Ochromonas sp. 3	6.3	—	—	0	4.3	7.2	1.6	3.3
Rhodomonas minuta	7.0	1.9	3.5	0	6.0	6.3	2.5	3.4
Microflagellates								
Gymnodinium varians	11.6	2.2	5.4	—	5.7	8.1	1.6	4.6
Gymnodinium helveticum	27.5	0	0	1.1	—	—	—	0.4
Ceratium hirudinella	44.8	—	—	—	0	1.7	0	0.6
Ciliates								
Strobilidium sp.	12.5	0.3	4.1	9.2	5.0	7.7	1.2	4.6
Urotricha sp.	14.0	0.3	1.5	5.9	6.3	4.1	1.9	3.3
Halteria sp.	18.0	—	—	3.8	—	—	1.0	2.4
Vorticella sp.	25.4	—	—	—	0	0	0	0
Strombidium viride	35.7	—	—	2.6	11.9	—	0.7	2.5
Strobilidium velox	36.5	0	—	—	7.6	—	0	2.5

* Equivalent spherical diameter (μm).

consistency in this pattern of protozoa mortality in our experiments occurred despite large changes in macrozooplankton community structure (0–69% *Daphnia* dominance) and thus tends to downplay the importance of consumer community composition in determining the fate of microbial production, although such alterations in consumers are influential for macrozooplankton grazing on bacteria in some ecosystems (Pace et al. 1990).

Unlike investigations of macrozooplankton grazing on phytoplankton (e.g. Lehman and Sandgren 1985), we did not observe a positive relationship between macrozooplankton biomass and growth for any protozoan taxon, and only one taxon was consistently unaffected by zooplankton grazing. These results suggest basic differences between the two protistan groups with respect to morphological adaptations and nutritional considerations.

First, unlike many algal species (Porter 1976), most planktonic protozoa do not possess thick gelatinous sheaths or persistent cell walls that might afford them viable gut passage. Second, the size range of the protozoa encountered here was much smaller than the distribution of ungrazed phytoplankton reported by Lehman and Sandgren (1985) and conformed to the size and shape of grazed phytoplankton from their studies. Third, the protozoa observed here did not possess elaborate morphologies, such as spines or extending appendages that are common among algal taxa (*see* Lee et al. 1985). However, *Vorticella* sp. was one of the few protozoan taxa resistant to grazing on all dates that it was encountered. As has been reported elsewhere (Pratt and Rosen 1983; Carrick and Fahnenstiel 1990), this taxon can grow attached to colonies of *Anabaena flos-aquae* (Lyngb.) deBribisson as was noted here, which apparently provided it refuge from potential grazers; colonies were large enough (>200-μm diam) to prevent handling by zooplankton (Lehman and Sandgren 1985).

Last, many of the protozoa we encountered were bacterivorous flagellates and ciliates, whose growth may not be directly augmented by nutrients excreted by mac-

rozooplankton (Taylor and Lean 1981), in contrast with phytoplankton (Lehman 1980). Thus, our results suggest that planktonic protozoa in Lake Michigan are dominated by small (<50-μm diam) unicellular forms that are susceptible to zooplankton grazing in that they generally do not possess morphological adaptations to afford resistance. Although protozoa may possess behavioral adaptations to avoid grazing, as indicated by the habits of *Vorticella* sp., those qualities were not assessed here.

Quantitative importance of the zooplankton-protozoan trophic link—The rate of phototrophic (Pnano and microflagellates) and heterotrophic (Hnano and ciliates) protozoan C consumption by macrozooplankton is similar to rates determined for Great Lakes phytoplankton (*see Table 2*). The existing conceptual model of trophic structure, at least in Lake Michigan, must be modified to account for these observations. Our estimates of heterotrophic protozoan C flux to macrozooplankton (0.3–11.2 μg C liter^{-1} d^{-1}) are comparable to flux rates of phytoplankton to zooplankton determined previously (from 2.1 to 15.8 μg C liter^{-1} d^{-1}) in the surface waters of Lake Michigan (Scavia and Fahnenstiel 1987). The flux of Hnano C alone accounted for >50% of the total heterotrophic transfer to zooplankton on most dates (mean, 2.1; range, 0.3–6.0 μg C liter^{-1} d^{-1}) and seems to indicate a tight coupling between Hnano and zooplankton as these flux estimates are comparable (on average 75%) to Hnano productivity values (mean, 2.7; range, 0.9–5.7 μg C liter^{-1} d^{-1}, Carrick 1990).

In addition, phototrophic protozoan (Pnano and microflagellates) flux rates (0.1–24.4 μg C liter^{-1} d^{-1}) were similar to those for algae, which suggests that the bulk of phototrophic C consumed by macrozooplankton in Lake Michigan is composed of flagellates, while a small fraction is composed of green algae, blue-green cyanobacteria, diatoms, and picoplankton. Thus, our results differ from contemporary food-web models in other freshwater systems (e.g. Weisse et al. 1990) in two ways. First, we observed comparable macrozooplankton grazing on both protozoa and phytoplankton; second, the bulk of heterotrophic nanoflagellate C appears to be consumed by macrozooplankton rather than smaller grazers such as ciliates.

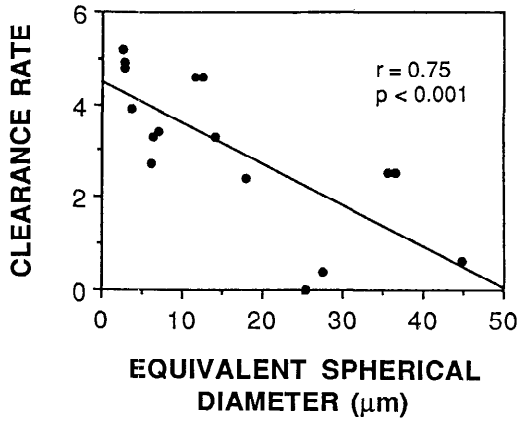

Fig. 4. Correlation between the average zooplankton clearance rates [ml (μg dry wt)$^{-1}$ d^{-1}] and the cell size of 16 dominant protozoan taxa.

The flux of C between heterotrophic protozoa and macrozooplankton has important implications for the fate of bacterial production in Lake Michigan. Heterotrophic protozoan (Hnano and ciliates) production (mean 10.8 μg C liter^{-1} d^{-1}, Carrick 1990) can account for >100% of bacterial production (mean, 25.7 μg C liter^{-1} d^{-1}; Scavia and Laird 1987), if we assume that these heterotrophic protozoa are bacterivores with a 30% growth efficiency. This observation, in concert with the high average flux of heterotrophic protozoa to macrozooplankton (mean, 3.9 μg C liter^{-1} d^{-1}), indicates that 15% of bacterial production in the lake can be consumed by macrozooplankton through a simple two-step trophic coupling. This estimate is probably conservative, as it does not account for direct bacterivory by macrozooplankton or mixotrophy among flagellates, which both can be significant (e.g. Sanders et al. 1989). Although this comparison is preliminary, our results demonstrate the importance of the microbial food web in Lake Michigan with implications for trophic structure in other aquatic systems.

The trophic importance of protozoa is illustrated further by the substantial fraction of potential macrozooplankton annual pro-

duction that can be supported by ingestion of protozoan C. If we assume production estimates (Borgmann et al. 1984) for Lake Ontario macrozooplankton (~2.0 μg C liter^{-1} d^{-1}) are in the range for production expected in Lake Michigan (macrozooplankton production has not been estimated for Lake Michigan) based on the similarities between lakes in terms of zooplankton composition and abundance, grazing on protozoa by macrozooplankton can support >80% of annual macrozooplankton production (assuming 15% zooplankton assimilation efficiency). These estimates indicate that protozoa are likely to be quantitatively significant prey for epilimnetic macrozooplankton in Lake Michigan.

Protozoa seem to be significant prey items for macrozooplankton in Lake Michigan, as clearance rates are similar to those previously determined for phytoplankton, particularly for protozoa <20 μm in equivalent spherical diameter. Also, a large fraction of potential annual zooplankton production in the lake can be accounted for by zooplankton grazing on protozoa. This observation, as well as the fact that the flux of heterotrophic protozoan C to macrozooplankton is comparable to that for phytoplankton, is contrary to contemporary pelagic food-web models (e.g. Weisse et al. 1990). These findings strongly imply that picoplankton C in Lake Michigan may be more directly transferred to higher trophic levels via a picoplankton-flagellate-zooplankton coupling, compared with the less direct picoplankton-flagellate-ciliate-zooplankton coupling proposed for oligotrophic marine systems (Sheldon et al. 1986).

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