The importance of microzooplankton versus phytoplankton to copepod populations during late winter and early spring in Lake Michigan

Marie H. Bundy, Henry A. Vanderploeg, Peter J. Lavrentyev, and Paul A. Kovalcik

Abstract: Feeding rates of the calanoid copepod *Leptodiaptomus sicilis* on natural assemblages of phytoplankton and microzooplankton were evaluated during late winter and early spring in Lake Michigan. Microzooplankton were the preferred food source for this copepod, and larger size fractions of phytoplankton were preferred to smaller size fractions. Ingestion rates of total chlorophyll *a* ranged from 2 to 14 ng·copepod^{-1}·day^{-1}, while ingestion rates of microzooplankton biomass ranged from 0.04 to 0.15 µg C·copepod^{-1}·day^{-1}. In these experiments, microzooplankton carbon accounted for 22%–74% of the total carbon ingested. Clearance rates of microzooplankton carbon were positively related to the larger size fractions of chlorophyll *a* and to total suspended solids. Measured ingestion rates of microzooplankton and phytoplankton carbon suggest that calanoid copepod populations have the potential to control microzooplankton production in late winter and early spring, and even with an abundance of phytoplankton carbon, food availability may limit the reproduction of *L. sicilis*. Because microzooplankton contribute significantly to the diet of these copepods, stimulation of the microbial food web by terrigenous inputs of nutrients and carbon may be transmitted to higher trophic levels (i.e., mesozooplankton and their predators) through heterotrophic flagellates and protozoans.

Résumé : Nous avons déterminé les taux d'alimentation du copépode calanoïde *Leptodiaptomus sicilis* sur les communautés naturelles de phytoplancton et de microzooplancton à la fin de l'hiver et au début du printemps au lac Michigan. Le microzooplancton constitue la source de nourriture préférée de ce copépode et les fractions de tailles plus grandes du phytoplancton sont choisies de préférence aux de tailles plus petites. Les taux d'ingestion de chlorophylle *a* varient de 2 à 14 ng·copépode^{-1}·jour^{-1}, alors que les taux d'ingestion de la biomasse du microzooplancton varient de 0,04 à 0,15 µg C·copépode^{-1}·jour^{-1}. Dans ces expériences, le carbone du microzooplancton représente de 22 à 74 % du carbone total ingéré. Les taux de clearance du carbone du microzooplancton sont en corrélation positive avec les fractions de tailles plus grandes de chlorophylle *a* et avec les solides totaux en suspension. Les taux d'ingestion de microzooplancton et de phytoplancton mesurés laissent croire que les populations de copépodes calanoïdes ont le potentiel de contrôler la production du microzooplancton à la fin de l'hiver et au début du printemps; même s'il y a une abondance de carbone du phytoplancton, la disponibilité de la nourriture peut restreindre la reproduction de *L. silicis*. Parce que le microzooplancton contribue de façon significative au régime alimentaire de ces copépodes, la stimulation du réseau alimentaire microbien par les apports de nutriments et de carbone d'origine terrestre peut se transmettre aux niveaux trophiques supérieurs (c'est-à-dire au mésozooplancton et à ses prédateurs) par l'intermédiaire des flagellés hétérotrophes et les protozoaires.

[Traduit par la Rédaction]

Introduction

Mesozooplankton assemblages of the glacial great lakes of North America, which extend from the Laurentian Great Lakes in the south to Great Bear Lake in the Canadian Arctic, are dominated by calanoid and cyclopoid copepods, especially in winter and early spring (Torke 2001; Barbiero et al. 2001). In Lake Michigan and many of the other lakes, cladocerans are dominant members of zooplankton assemblages in spring and fall (e.g., Torke 1975, 2001; Barbiero et al. 2001). The winter and spring isothermal period is important to calanoids because it is a major reproductive period (e.g., Torke 1975). Calanoids have traditionally been considered to be food-limited in late winter because developmental

Received 21 May 2003. Accepted 31 December 2004. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 28 September 2005.
J17538

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 doi: 10.1139/F05-111

rates, feeding rates, storage of energy reserves, and reproduction during the spring isothermal period respond positively to increases in phytoplankton biomass and changes in food quality (Vanderploeg et al. 1992*a*). Overwintering females rely heavily on stored lipids for reproduction, and can exist for long periods at extremely low food levels. However, the ability to maintain reproductive output throughout the spring depends on their ability to replenish lipids that are depleted during the initial reproductive effort (Vanderploeg et al. 1992*a*, 1992*b*). Therefore, population success of the copepods that provide food for planktivorous fish during spring and summer is a function of the quantity and quality of food that reproducing females and maturing juveniles encounter in late winter and early spring.

During the late winter – early spring in Lake Michigan, phytoplankton assemblages are dominated by centric diatoms and cryptophytes. These phytoplankton increase in abundance until primary production peaks and then decline as stratification occurs and nutrients are depleted in the upper mixed layer (e.g., Fahnenstiel and Scavia 1987; Barbiero and Tuchman 2001). Although food webs in large lakes have been traditionally been considered to be controlled by autotrophic processes, recent data indicate that, during a large portion of their annual cycle, they may be driven by allochthonous organic inputs and resuspension, rather than in situ primary production (Cotner and Biddanda 2002). Therefore terrigenous organic inputs and associated heterotrophic production can be as important as primary production in the cycling of lake carbon (Grey et al. 2001). The spring diatom bloom has been identified as fuel for the pelagic food web, providing a boost to copepod populations as they begin to feed after overwintering. However, calanoids may enter their peak reproductive period before the spring bloom, when phytoplankton biomass is relatively low (Vanderploeg et al. 1992*a*). Studies conducted over the last two decades suggest that, in large temperate lakes like Lake Michigan, the microbial food web controls carbon transfer during this time (e.g., Carrick et al. 1991; Fahnenstiel et al. 1998; Biddanda and Cotner 2002).

Because heterotrophic protists can provide an important link between microbial food webs and higher trophic levels (e.g., Carrick et al. 1991; Lampman and Makarewicz 1999), information about mesozooplankton feeding on autotrophic versus heterotrophic components of the seston is critical to understanding pathways of energy transfer and the potential contribution of the microbial food web to productivity at higher trophic levels. There is a shortage of information about the relative contribution of microzooplankton versus phytoplankton to copepod diets in large temperate lakes, and little is known about the influence of different phytoplankton size fractions and taxa on copepod consumption of microzooplankton (Burns and Gilbert 1993). It is also unclear whether or not copepod selective feeding can control rates and pathways of carbon transfer by removing a significant amount of either phytoplankton or microzooplankton biomass.

This work was carried out as part of the National Science Foundation – National Oceanic and Atmospheric Administration Episodic Events – Great Lakes Experiment (NSF – NOAA EEGLE), and experiments were conducted simultaneously with EEGLE oceanographic survey cruises along established transects running perpendicular to the shore in southern Lake Michigan (Fig. 1). The goal of the EEGLE project was to investigate effects on the ecology of Lake Michigan of late winter – early spring storm events that resuspend sediment particles and associated nutrients and toxins (Eadie et al. 1996; Robbins and Eadie 1991). By stimulating heterotrophic production in nearshore regions of the lake, episodic physical processes, such as resuspension or increased runoff that locally increase terrigenous inputs of carbon and nutrients, can be important to the production of higher trophic levels. The pathways for this increased production may move energy from the microbial food web through copepods to larval and adult planktivorous fish.

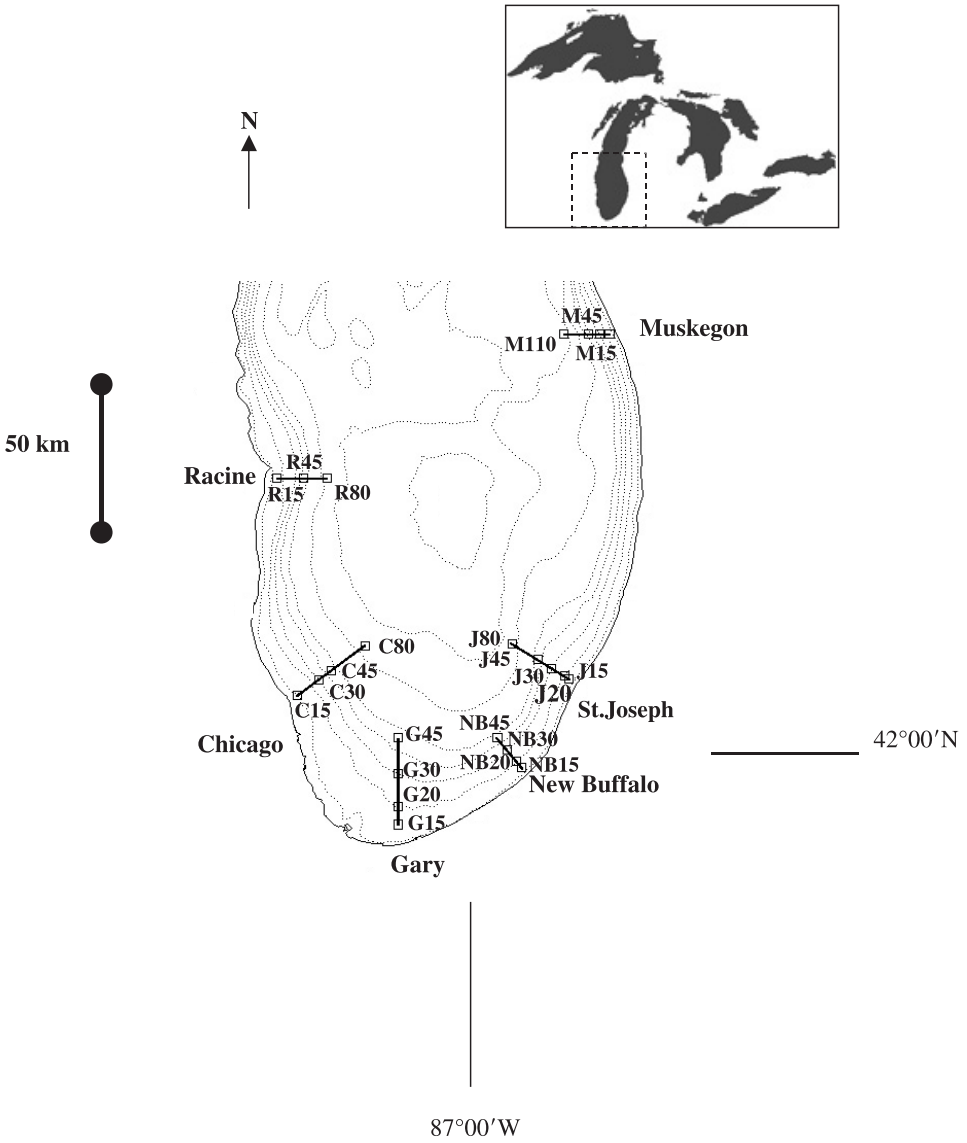
To reach a better understanding of the potential for resuspension events to affect the lower levels of the pelagic food web, we conducted copepod feeding experiments in 1998–2000 using natural Lake Michigan seston. Here we focus on the feeding responses of the calanoid *Leptodiaptomus sicilis* because this copepod is an omnivore that occurs in all of the Great Lakes, it is one of the dominant calanoids in Lake Michigan during late spring – early winter (Robertson 1966; Barbiero et al. 2001), and it is a primary prey item for planktivorous fish (e.g., Evans 1990). This species is well adapted to low temperatures. One of the authors (H.A. Vanderploeg) has examined feeding and reproduction of *L. sicilis* in a number of environments during the winter at Great Lakes sites, as well as under ice cover at Great Slave Lake in the Canadian subarctic. These copepods feed and reproduce at temperatures just above 0 °C at very low particle concentrations. Our goal was to investigate the potential for copepods to utilize microzooplankton and phytoplankton biomass in natural plankton communities during late winter and early spring, and to examine the possibility that copepod populations can control microzooplankton production during this physically dynamic time when storm-induced resuspension events frequently occur.

Methods

Water, zooplankton for experiments, physical and biochemical data, and zooplankton abundance data were collected from stations along EEGLE survey transects (Fig. 1; Table 1). All experiments were conducted between February and April before water column thermal stratification occurred. At each station, conductivity, temperature, and depth were measured using a Sea-Bird Electronics Sealogger Profiler (model No. SBE 25; Sea-Bird Electronics, Inc. Bellevue, Washington, USA). Dry weight of total suspended solids (TSS) was determined gravimetrically from discrete water samples collected at each experimental site.

Zooplankton were collected using a large aspect ratio (0.5 m diameter, 2.25 m length) 202- or 303-μm mesh plankton net with a solid cod end that was slowly towed vertically to the surface from 10 m above the bottom. Water for experiments was collected 10 m below the surface using a 30-L Niskin bottle that was slowly and gently emptied into polycarbonate carboys through a submerged hose. In some cases, water was gently screened through a submerged 400-μm mesh net to remove stray metazoans such as

Fig. 1. Location of stations (□) and transects (solid lines) of National Science Foundation – National Oceanic and Atmospheric Administration Episodic Events – Great Lakes Experiment survey cruises in Lake Michigan. Dotted lines, depth contour internals (10 m). Square in inset shows location of the study area in the Great Lakes region.



copepods and copepodites, while in other cases, metazoans were removed by pipetting.

Zooplankton were maintained at close to ambient temperatures in insulated containers until grazing experiments commenced. Adult female cephalothorax length, body volume, and dry weight are approximately 1.2 mm, 1.0 µL, and 30 µg, respectively (Vanderploeg et al. 1992*b*). Carbon content is approximately 45% for calanoids (Mauchline 1998). Grazing experiments were conducted at ambient temperatures under dim light and a 12 h light – 12 h dark regime. Nine 1180- or 610-mL Pyrex bottles were slowly filled with water from the site, and non-gravid *L. sicilis* females were individually pipetted into four of the bottles for a final concentration of 17–34 copepods·L^{-1} (mean concentration was 29.4 copepods·L^{-1}). Controls consisted of three bottles that were identical to experimental bottles but without added copepods. Three initial bottles were treated the same as the controls were, but were sampled for phytoplankton and microzooplankton approximately 1 h after all the bottles were filled. Bottles were sealed without bubbles and placed on a plankton wheel that rotated at 0.25 revolutions·min^{-1}. Nutrients were not augmented in these experiments because a preliminary experiment that compared final chlorophyll *a* (Chl *a*) concentrations in grazing bottles with and without added nutrients indicated that zooplankton excretion was not a significant factor influencing phytoplankton growth. An isotope-addition experiment confirmed these results (W.S. Gardner, The University of Texas at Austin, Marine Science Institute, 750 Channel View Drive, Port Aransas, TX 78373-5015, USA, personal communication). After 24 h, copepods were pipetted from grazing bottles and samples were collected from each control and grazing bottle for size-fractionated Chl *a* (Bowers 1980; Vanderploeg et al. 2001) and microzooplankton enumeration. The 1998 and February

Table 1. Station data.

Date	Station	TSS ($mg \cdot L^{-1}$)	Temperature (°C)	Chl *a* size fraction	Chl *a* ($\mu g \cdot L^{-1}$)	Phytoplankton carbon > 5 µm from cell counts ($\mu g \cdot L^{-1}$)	Picophytoplankton carbon ($\mu g \cdot L^{-1}$)	Phytoplankton carbon : Chl *a*	Initial microzooplankton carbon ($\mu g \cdot L^{-1}$)
1 April 1998	M110	1.70	4.0	>53	0.28	14.0	n/a	n/a	2.0
				>10–53	0.26				
				<10	0.73				
				Total	1.27				
25 February 1999	M45	1.00	1.5	>53	0.19	n/a	9.5	n/a	9.1
				>10–53	0.13				
				<10	0.78				
				Total	1.10				
13 March 1999	J60	0.60	1.0	>53	0.27	n/a	21.2	n/a	8.6
				>10–53	0.12				
				<10	0.93				
				Total	1.32				
7 April 1999	C80	1.80	3.0	>53	0.44	14.0	9.0	13.1	8.8
				>10–53	0.24				
				<10	1.08				
				Total	1.75				
20 February 2000	J80	1.10	2.0	>53	0.45	8.5	15.4	19.0	6.9
				>10–53	0.21				
				<10	0.60				
				Total	1.26				
22 February 2000	G45	1.00	2.0	>53	0.35	8.5	19.2	22.9	6.9
				>10–53	0.22				
				<10	0.87				
				Total	1.96				
11 March 2000	J30	3.00	2.0	>53	0.50	13.7	11.7	21.2	8.7
				>10–53	0.13				
				<10	0.57				
				Total	1.20				
12 March 2000	NB20	4.40	3.0	>53	0.71	45.4	3.3	24.4	5.6
				>10–53	0.28				
				<10	1.01				
				Total	2.00				
13 March 2000	C80	1.10	2.0	>53	0.68	13.3	8.4	11.9	5.6
				>10–53	0.25				
				<10	0.89				
				Total	1.82				

Note: TSS, total suspended solids; n/a, not applicable.

and March 1999 experiments were conducted in walk-in environmental rooms at the NOAA Great Lakes Environmental Research Laboratory (GLERL) with water and zooplankton that were collected within 24 h. All other experiments were conducted in incubators on shipboard.

For microzooplankton samples, initial and final 100-mL samples were removed from each bottle, preserved in 1% acid Lugol's iodine, and stored at 4 °C until they were counted. Triplicate 50- to 100-mL subsamples were settled in an Utermöhl chamber (Utermöhl 1958), and the entire chamber was then counted under an Olympus IX-70 DIC (differential interference contrast) inverted microscope. To estimate microzooplankton biomass, the linear dimensions of 30–90 individuals (fewer for less abundant taxa) were measured at 400×–1000× and converted to volumes using the appropriate geometric shapes. The volume of tintinnid cells was determined using the same approach as that used for aloricate protists, since they were clearly visible inside their loricas under differential interference contrast microscopy.

To estimate microzooplankton biomass in grazing experiments, we used the volume-to-carbon regression of Putt and Stoecker (1989) for ciliates. Their data were obtained using the same fixative as that used in this study (Lugol's iodine), as well as ciliate species that were similar to the predominant taxa in Lake Michigan. The regression includes a correction of about 20% for cell shrinkage due to preservation. Recent studies indicate that some ciliates can shrink by as much as 40% in Lugol's iodine (e.g., Chaput and Carrias 2002). Therefore our estimates of ciliate carbon may be conservative. For dinoflagellates, we used the equations from Menden-Deuer and Lessard (2000) to convert volume to carbon. Such equations have been shown to be more appropriate for estimating freshwater plankton biomass than those considering all protist groups as a whole (Gosselain et al. 2000). These dinoflagellate volume estimates were corrected assuming 30% shrinkage (Montagnes et al. 1994).

During the April 1999 experiment and all of the 2000 experiments, samples were taken from initial bottles and preserved in Lugol's solution so that the abundance and species composition of ambient phytoplankton assemblages could be related to clearance rates of Chl *a* size fractions. For the April 1999 experiment, phytoplankton samples were collected at the site 2 days prior to the experiment. Subsamples (10- to 50-mL) were filtered onto membrane filters for permanent mounting on slides. Phytoplankton >5 μm were identified to the lowest practical taxonomic unit (generally to species or genus). Phytoplankton were also classified by broad taxonomic group (i.e., diatoms, flagellates, cryptophytes, chlorophytes, chrysophytes, and cyanobacteria) and by biovolume: cells that were >1000, 1000–200, and <200 μm^3 were designated as large, medium, and small, respectively.

Photosynthetic picoplankton (in this case, cells <3 μm, which were mostly single-celled *Synechococcus*-like cyanobacteria) were preserved with 2% paraformaldehyde, filtered onto 0.2-μm pore size black polycarbonate membrane filters, and frozen at –20 °C immediately after collection until counted via epifluorescence microscopy (MacIsaac and Stockner 1993). Picoplankton cells were sized using Image Pro 4.5 image analysis software and a SPOT-2 digital camera. At least 500 cells were measured in each sample. Cell volumes were converted to carbon based on a conversion factor for cold-water picoplankton of 0.21 pg C·μm^{-3} (Booth et al. 1993).

Grazing rates on phytoplankton were determined by measuring the disappearance of chlorophyll size fractions (>53, 10–53, and <10 μm) in experimental bottles. Using the disappearance of chlorophyll to measure grazing is a common method that provides results similar to short-term ingestion experiments using radiolabeled algae (Hargis 1977). Strictly speaking, the method measures assimilation, and problems could arise with digestive-resistant algae; however, digestive-resistant algae were not part of the winter or spring algal assemblage that was examined here. Excluding phaeopigments from the analyses reduced the potential for fluorescence from pigments in fecal material to bias calculations of ingestion and clearance rates. The use of size-fraction analyses also provided a means of determining feeding and selectivity (measured as relative clearance rates, sensu Vanderploeg 1994) of different size classes of algae.

Size-fractionated Chl *a* was analyzed by filtering two replicate subsamples (usually 180 mL) of water from initial, control, and grazing bottles through an apparatus containing a set of stacked Nitex screens and a GF/F filter immersed in water (Bowers 1980; Vanderploeg et al. 2001). The largest phytoplankton fraction was collected on a 53-μm mesh screen followed by a 10-μm mesh screen, and then a GF/F filter (nominal pore size, 0.7 μm). Little flocculation was noted in the jars, so retention of smaller cells on the larger meshes most likely did not bias our measurements (Logan et al. 1994). Screens and filters were folded and inserted into plastic test tubes, then frozen and later extracted with *N*,*N*-dimethylformamide and analyzed fluorometrically (Speziale et al. 1984). Total Chl *a* was obtained from the sum of the three size fractions.

In experiments like these, there is always the risk of preferred prey being removed to a greater degree than other prey, thus altering the relative concentrations over the experimental period. Concentrations of Chl *a* were always greater than 44% of original concentrations for all size fractions in all experiments except the 12 March 2000 experiment, in which the 10–53 μm fraction fell to 34% of the original concentration. Microzooplankton were severely depleted in some cases. In some studies, this relative depletion of preferred prey resulted in the underestimation of clearance rates of phytoplankton and microzooplankton carbon (e.g., Zeldis et al. 2002). However, for *L. sicilis*, unless a prey is completely eliminated, it is unlikely that changing relative prey concentrations will greatly affect clearance rates, and therefore copepod selectivity, if concentrations of prey are initially below the incipient limiting concentration. Feeding experiments similar to these that used large and small phytoplankton prey in bottle experiments show that selectivity of *L. sicilis* does not change over a broad range of relative concentrations (Vanderploeg et al. 1984). Selectivity and feeding experiments conducted with long- and short-term exposure of diaptomids to algae with radiolabeled carbon and phosphorus (Demott 1990; Demott 1995) showed that, in situations in which there was depletion of preferred prey over longer incubations, long- and short-term experiments provided much the same results, with no evidence that short-term exposure provided higher clearance rates.

Clearance rates and ingestion rates of each size fraction of Chl *a* were calculated using Frost's equations (Frost 1972). Copepod ingestion and clearance rates when feeding on microzooplankton were calculated from initial and final microzooplankton biomass according to Gifford (1993). So that we could make general comparisons between this and other published studies and relate the autotrophic biomass to heterotrophic biomass that was consumed by copepods during this study, Chl *a* concentrations in each size fraction were converted to carbon using a carbon : Chl *a* ratio of 20. This ratio was derived from biomass estimates from >5-μm cell counts that were made during the April 1999 and March–April 2000 experiments, which were compared with Chl *a* in the total size fraction. Cell dimensions of different taxa were converted to cell volume and then to carbon from estimates derived from empirical studies for diatoms (Strathmann 1967) and non-diatoms (Verity et al. 1992). Conversion equations from these studies used non-linear regression models to account for higher carbon content per unit volume in smaller cells, and experiments included a wide range of cell sizes. These two models produce similar results to regression models derived from experimental data combined with data from literature studies (Menden-Deuer and Lessard 2000). However, the model of Strathmann (1967) may underestimate carbon by 20% for diatoms less than 30 μm^3 in biovolume and may overestimate carbon by 5%–60% as diatoms increase in biovolume from 300–10^6 μm^3 (Menden-Deuer and Lessard 2000). The dominant diatom species in the present study were found in the medium and small size classes, in which biovolumes were in the range of 20–800 μm^3. Therefore, algal carbon contained in the larger size fractions may have been overestimated when large diatoms dominated the phytoplankton community. By including the biomass of picocyanobacteria at each station (which ranged from 3.3 to 21.2 μg $C \cdot L^{-1}$; Table 1), an average ratio of 18.8 for all experiments was obtained. For our general comparisons, we rounded the ratio to 20. Our empirically derived carbon : Chl *a* ratio approximates the ratio that can be derived from data collected at similar temperatures in an earlier study in Lake Michigan in early spring (Fahnenstiel and Scavia 1987, their fig. 2), and is also within the range empirically derived for estuarine phytoplankton (Gallegos and Vant 1996).

In addition to directly affecting phytoplankton concentrations in experimental bottles through grazing, mesozooplankton indirectly affect phytoplankton concentrations by feeding on microzooplankton grazers and thereby reducing this component of mortality (Nejstgaard et al. 2001). During the 2000 experiments, dilution experiments (Landry 1993) were conducted to assess the grazing impact of microzooplankton on phytoplankton assemblages. Water used in dilution experiments was collected at the same time as water used in copepod grazing experiments. The size fractions examined in dilution experiments were 25, 5, and 0.4 μm. The correction factor (k_p) for the effects of copepod predation on microzooplankton grazers, was derived according to the methods of Nejstgaard et al. (2001):

$$g_{\mathrm{corr},p} = g_{\mathrm{cop},p} + k_p \quad (1)$$

$$k_p = g_{\mathrm{mic},p}(\hat{c} - \hat{c}^*)\hat{c}^{-1} \quad (2)$$

$$\hat{c} = (c_t - c_0)(\ln(c_t/c_0))^{-1} \quad (3)$$

$$\hat{c}^* = (c_t^* - c_0)(\ln(c_t^*/c_0))^{-1} \quad (4)$$

where $g_{\mathrm{corr},p}$ is the corrected copepod grazing coefficient on a specific size fraction p; $g_{\mathrm{cop},p}$ is the uncorrected copepod grazing coefficient; $g_{\mathrm{mic},p}$ is the microzooplankton grazing coefficient determined from dilution experiments; $\hat{c}$ and $\hat{c}^*$ are microzooplankton biomass in the control and grazing bottles, respectively, at the end of the experiment; c_t is microzooplankton biomass at time t; and c_0 is the initial microzooplankton biomass.

For the 1998 and 1999 grazing experiments, in which concurrent dilution experiments were not conducted, the average value from the 2000 experiments was used as an estimate of $g_{\mathrm{mic},p}$. In all experiments, the g_{mic} determined for the <25-μm fraction of Chl *a* in dilution experiments was used to correct copepod grazing on the <53-μm fraction. Because more than 85% of the microzooplankton present during experiments were less than 60 μm in the longest dimension, no correction was used for the >53-μm fraction because it was assumed that the highest feeding rates of the majority of microzooplankton were primarily for phytoplankton cells smaller than their longest dimension.

Statistics

To test for the effect of microzooplankton biomass and each size fraction of Chl *a* on copepod clearance rates, each station–date combination was treated as an independent sample ($n = 9$, with three or four replicates at each size fraction for a clearance rate of Chl *a*, and three or four replicates of clearance rate of microzooplankton biomass per experiment; Table 1). Levine's test showed that clearance rates were significantly heteroscedastic with respect to date. Therefore, we log-transformed clearance rate data and conducted an analysis of variance (ANOVA; SAS Version 7: Proc GLM; Potvin and Roff 1993) to examine the direct effects of food category (i.e., the three size fractions of Chl *a* and microzooplankton carbon) and sample date on copepod clearance rate. Type-III sum of squared error was used for the interpretation of results. Unless otherwise noted, a posteriori comparisons were made using the Newman–Keuls procedure (SNK test).

Spearman correlation analyses were also used to explore individual relationships among the following: (*i*) clearance rates of each size fraction of Chl *a* with TSS, temperature, initial Chl *a* in each size fraction, initial microzooplankton carbon, and clearance rates of microzooplankton carbon; (*ii*) clearance rates of microzooplankton carbon with TSS, temperature, clearance rates of total Chl *a*, initial microzooplankton carbon, and initial Chl *a* concentration; (*iii*) initial concentrations of total Chl *a* with initial microzooplankton carbon, TSS, and temperature; and (*iv*) initial microzooplankton carbon with TSS and temperature. A sequential Bonferroni-type procedure was used to control the false-discovery rate for independent statistics, as described in Benjamini and Hochberg (1995).

Results

Total initial phytoplankton biomass, estimated from the sum of initial carbon in each size fraction, ranged from 22.0

Fig. 2. Initial microzooplankton and phytoplankton biomass (μg C·L^{-1}) for each experiment. Shaded bars, microzooplankton; solid bars, phytoplankton.

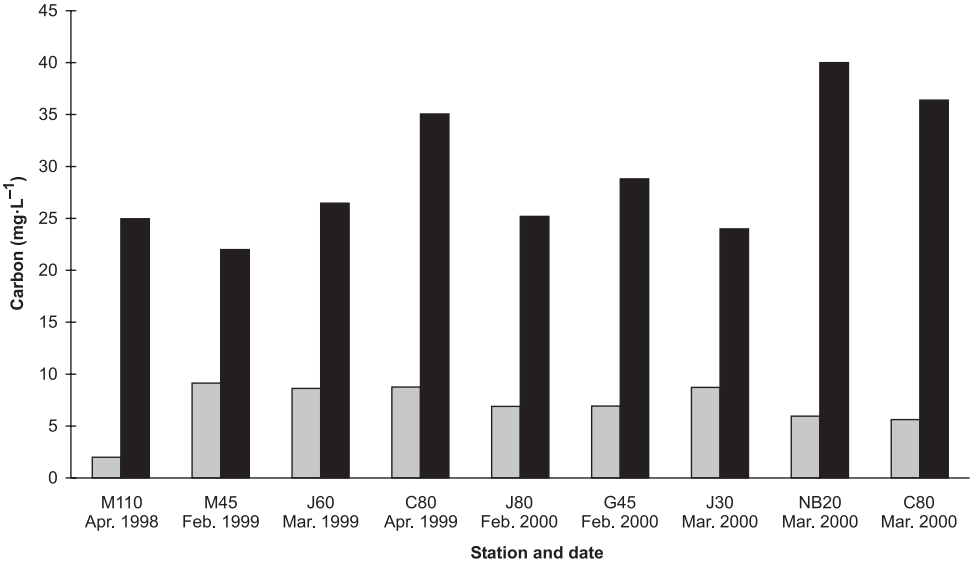
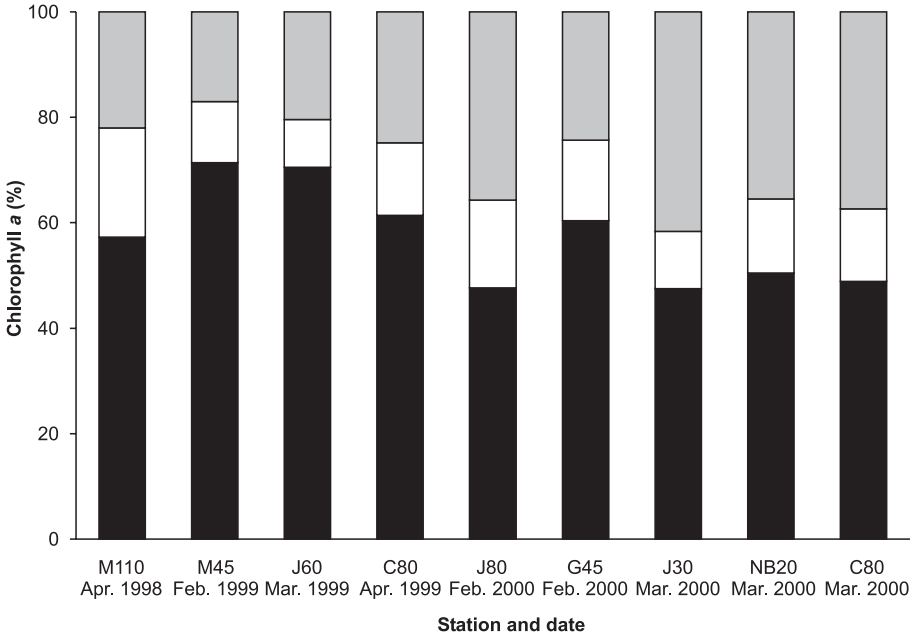


Fig. 3. Size distributions of initial chlorophyll *a* for each experiment, as percentage of total Chl *a*. Shaded bars, >53 μm; open bars, 10–53 μm; solid bars, <10 μm.

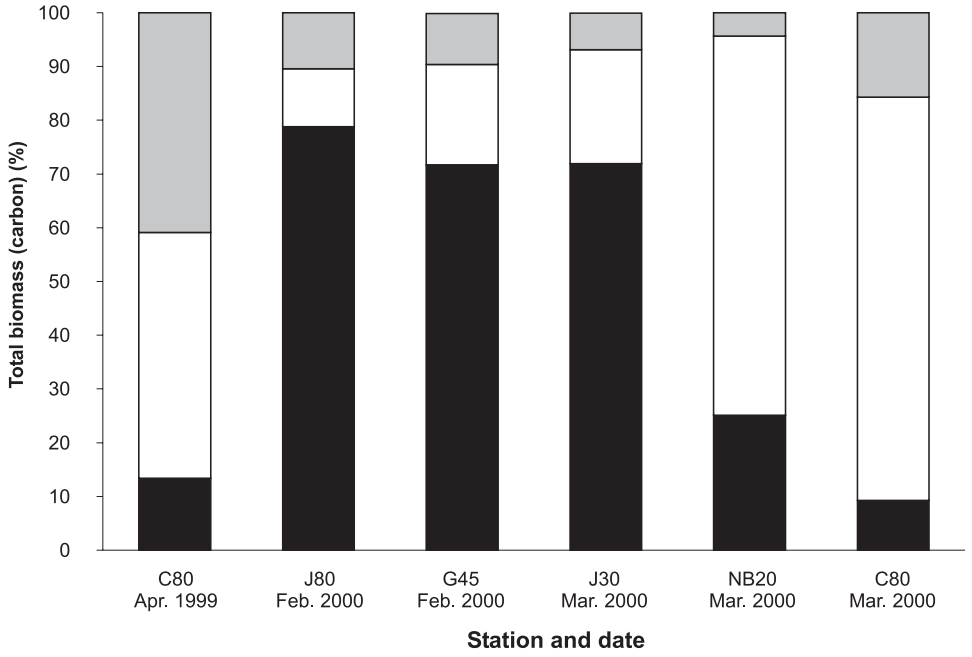


to 40.0 μg C·L^{-1}, while initial microzooplankton biomass ranged from 2.0 to 9.1 μg C·L^{-1} (Table 1; Fig. 2). When data from all experiments were pooled, the <10-μm fraction was the largest fraction of initial Chl *a* in all experiments (ANOVA, $p < 0.001$; SNK a posteriori test, $p < 0.05$), making up more than 50% of Chl *a*, and there were no significant differences in Chl *a* concentration between the 10–53 and the >53-μm fractions. When experiments were considered separately, the <10-μm fraction was always qualitatively the largest fraction of Chl *a* (more than 50% of the total), and the >53-μm fraction was larger than the 10–53 μm fraction (Fig. 3). Biomass (carbon) was also dominated by phytoplankton (Fig. 2).

The available data from microscopic counts of >5-μm phytoplankton showed that the <10-μm fraction, which was the largest fraction of Chl *a* in each of these experiments (Fig. 3), was numerically dominated by nanoflagellates, cyanobacteria, small diatoms, and chlorophytes, although species composition varied among experiments. Diatoms and cryptophytes composed most of the 10–53 μm fraction, and diatoms composed the >53-μm fraction.

The size fractions that accounted for most of the biomass varied among experiments (Fig. 4), as did the dominant taxa. In contrast with other stations where small cells accounted for most of the biomass, the dominant species at NB20 and C80 during March 2000 was the cryptophyte *Cryptomonas*

Fig. 4. Taxonomic composition of initial phytoplankton assemblage for each experiment in which cell counts were conducted, shown as a percentage of total biomass (carbon). Shaded bars, large cells; open bars, medium cells; solid bars, small cells.



erosa (~1800 μm^3), which made up approximately 60% of the biomass. At C80 in April 1999, single-celled (*Synedra ulna*) and chain-forming and colonial diatoms (e.g., *Tabellaria fenestra* and *Melosira italica*) were the other dominant taxa in the >53-μm fraction in most other experiments. In the March 2000 J30 experiment, the chlorophyte *Gloeocystis planktonica* (~100 μm^3) was abundant (~28% abundance and 24% biomass). Picophytoplankton (mostly *Synechococcus*-like cyanobacteria) were abundant at all stations, and their biomass ranged from 3.3 to 21.2 μg C·L^{-1} (Table 1). The dominant microzooplankton taxa were ciliates (tintinnids, oligotrichs, prostomatids, and scuticociliates), with most cells in the range of 1500 – 60 000 μm^3, and a heterotrophic dinoflagellate, *Gymnodinium helveticum* (19 600 μm^3). Direct counts to quantify changes in microzooplankton species distributions during feeding experiments showed that oligotrichs and tintinnids in the 30- to 50-μm equivalent spherical diameter (ESD) range were most vulnerable to copepod predation (Kovalcik 2001).

When experiments were pooled, copepod clearance rate of microzooplankton carbon was significantly higher than the clearance rate of any size fraction of Chl *a* (ANOVA on rank-transformed data, $p < 0.0001$; SNK a posteriori test, $p < 0.05$; Fig. 5*a*). Clearance rate of the 10–53 μm fraction was significantly higher than clearance rate of the >53-μm and the <10-μm fraction (SNK a posteriori test, $p < 0.05$; Fig. 5*a*).

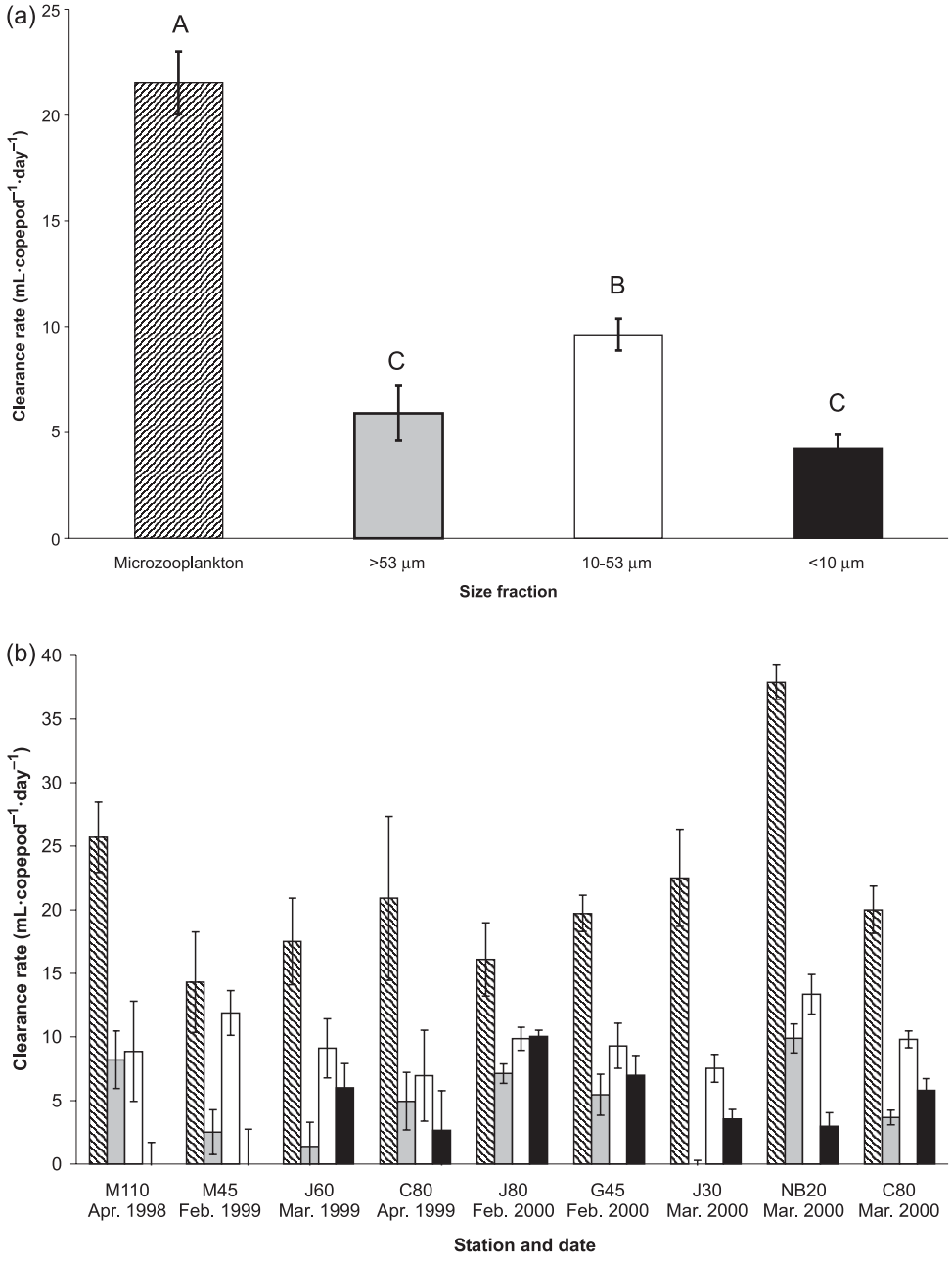
Clearance rates of microzooplankton carbon ranged from 14.3 (M45, February 1999) to 37.0 mL·copepod^{-1}·day^{-1} (NB20, March 2000; Table 2). When the experiments were considered separately, clearance rates of copepods feeding on microzooplankton were always among the highest of all food categories, and clearance rates of the 10–53 μm fraction were always equivalent or next highest (Table 3; Fig. 5*b*). Clearance rates of the preferred size fraction of Chl *a* (i.e., the fraction for which clearance rates were highest; Vanderploeg et al. 1984) ranged from 7.0 (C80, April 1999) to 13.4 mL·copepod^{-1}·day^{-1} (NB20, March 2000). At the end of the experiments, concentrations of the most preferred phytoplankton size fraction (10–53 μm) averaged 62% of initial concentrations, while the concentrations of the <10-μm and >53-μm size fractions averaged 80% and 83%, respectively. In some instances, microzooplankton prey were depleted from some of the experiments, and the concentration at the end of the experiments was, on average, 22% of initial concentrations.

Ingestion rate total Chl *a* ranged from 2 (J30, March 2000) to 14 ng·copepod^{-1}·day^{-1} (NB20, February 2000) or from 40 to 28 ng C·copepod^{-1}·day^{-1}, respectively (Table 2). Ingestion rates of microzooplankton biomass ranged from 0.04 μg (M110, April 1998) to 0.15 μg C·copepod^{-1}·day^{-1} (C80, April 1999; Table 2). Microzooplankton carbon accounted for between 22% and 74% of the carbon ingested (i.e., total phytoplankton carbon ingested + microzooplankton carbon ingested) across all experiments (Fig. 6).

There was a significant positive relationship ($p < 0.05$) between clearance rates of microzooplankton carbon and initial concentrations of Chl *a* in the 10–53 μm fraction (Spearman's rho = 0.70, $n = 9$), TSS (Spearman's rho = 0.82, $n = 9$), and temperature (Spearman's rho = 0.76, $n = 9$). There was also a significant relationship between clearance rates of the >53-μm size fraction of Chl *a* and temperature (Spearman's rho = 0.80, $n = 9$).

There were no significant relationships between clearance rates of any other size fraction of Chl *a* and TSS, temperature, initial Chl *a* in any size fraction, initial microzooplankton carbon, or clearance rates of microzooplankton carbon. There were no significant relationships between ingestion rates of total Chl *a* and TSS or temperature. There were also no significant relationships between initial Chl *a* concentra-

Fig. 5. Clearance rates (mL·copepod^{-1}·day^{-1} ± standard error) of microzooplankton and Chl *a* size fractions. (*a*) All experiments considered together. Letters (A > B > C) indicate ranking of clearance rates (analysis of variance, $p < 0.0001$; Newman–Keuls procedure, $p < 0.05$). (*b*) Individual experiments. Hatched bars, microzooplankton; shaded bars, >53 µm; open bars, 10–53 µm; solid bars, <10 µm.



tion or initial microzooplankton biomass and TSS or temperature, or between TSS and temperature ($p > 0.05$ for all non-significant comparisons).

Discussion

Selective feeding

Leptodiaptomus sicilis, like many calanoids, is a highly selective feeder, and feeding rates on prey that are not the preferred size or shape can be an order of magnitude lower than those on preferred prey (Vanderploeg et al. 1988; Vanderploeg 1994). In general, *L. sicilis* selects prey that are larger than 10 µm ESD (Vanderploeg et al. 1992*a*). The smallest particles *L. sicilis* typically feeds upon are approximately 3 µm ESD, which are captured passively and without remote detection (Vanderploeg and Paffenhöfer 1985). The diameter of the mouth most likely limits ingestion of whole cells to those less than 30 µm in diameter. Feeding preferences for larger (>10 µm) phytoplankton have been observed in natural seston (Bowers 1980; Vanderploeg et al. 1984), and rates as high as 41.5 mL·copepod^{-1}·day^{-1} have been observed for diaptomids feeding on elongated diatoms (~100 µm in longest dimension; Vanderploeg et al. 1988). In the present study, the highest clearance rates on phytoplankton were observed for the 10–53 µm size fraction. Large- and mid-sized diatoms that are elongated in one di-

Table 2. Clearance rates and ingestion rates.

Date	Station	Chl *a* size fraction	Chl *a* clearance rate (mean ± SE; mL·copepod·day^{-1})	Chl *a* ingestion rate (ng·copepod^{-1}·day^{-1})	Phytoplankton carbon ingestion rate (µg·copepod·day^{-1})	Microzooplankton carbon clearance rate (mean ± SE; mL·copepod·day^{-1})	Microzooplankton carbon ingestion rate (µg·copepod·day^{-1})
1 April 1998	M110	>53	8.20±1.8	2.30	0.05	25.4±4.0	0.04
		>10–53	8.86±1.8	2.33	0.05		
		<10	0.00	0.00	0.00		
		Total		4.63	0.09		
25 February 1999	M45	>53	2.52±1.9	0.47	0.01	14.3±3.4	0.12
		>10–53	11.88±2.3	1.51	0.03		
		<10	0.00	0.00	0.00		
		Total		1.98	0.04		
13 March 1999	J60	>53	3.76±2.3	1.02	0.02	17.5±6.4	0.13
		>10–53	9.11±3.6	1.09	0.02		
		<10	6.01±3.1	5.61	0.11		
		Total		7.72	0.15		
7 April 1999	C80	>53	4.94±0.8	2.15	0.04	20.9±2.9	0.15
		>10–53	6.95±0.9	1.68	0.03		
		<10	2.66±0.5	2.86	0.06		
		Total		6.69	0.13		
20 February 2000	J80	>53	7.11±1.6	3.20	0.06	16.1±1.4	0.09
		>10–53	9.85±1.8	2.07	0.04		
		<10	10.05±1.6	6.03	0.12		
		Total		11.30	0.23		
22 February 2000	G45	>53	0.30±0.3	0.11	0.00	19.7±3.8	0.12
		>10–53	9.29±1.1	2.04	0.04		
		<10	7.09±0.8	6.17	0.12		
		Total		8.32	0.17		
11 March 2000	J30	>53	–0.53±1.2	0.15	0.00	22.5±1.4	0.14
		>10–53	7.52±1.6	0.98	0.02		
		<10	3.56±1.1	2.03	0.04		
		Total		3.16	0.06		
12 March 2000	NB20	>53	9.88±0.6	7.01	0.14	37.0±1.9	0.14
		>10–53	13.36±0.7	3.74	0.07		
		<10	2.96±0.9	2.99	0.06		
		Total		13.75	0.27		
13 March 2000	C80	>53	3.67±0.3	2.50	0.05	20.0±2.6	0.09
		>10–53	9.80±0.9	2.45	0.05		
		<10	5.80±0.5	5.16	0.10		
		Total		10.11	0.20		

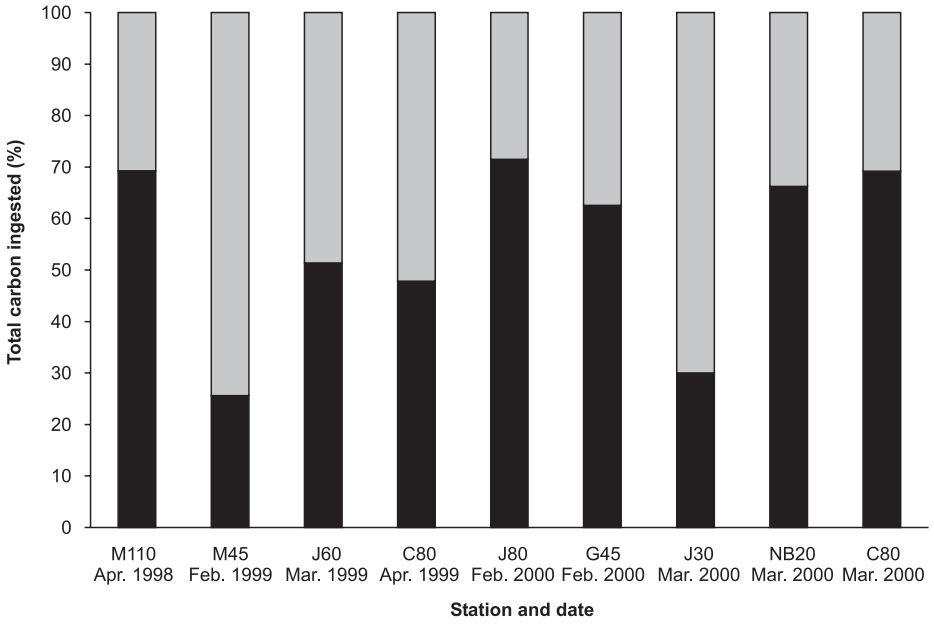
Note: SE, standard error.

Table 3. Analysis of variance (ANOVA) results for each experiment.

Date	Station	Microzooplankton	Chl *a* size fraction >53 μm	10–53 μm	<10 μm	*p* value
1 April 1998	M110	A	B	B	C	0.0001
25 February 1999	M45	A	B	A	B	0.001
13 March 1999	J60	ns	ns	ns	ns	ns
7 April 1999	C80	A	B	B	C	0.0001
20 February 2000	J80	A	B	AB	AB	0.05
22 February 2000	G45	A	C	B	B	0.0001
11 March 2000	J30	A	D	B	C	0.0001
12 March 2000	NB20	A	C	B	D	0.0001
13 March 2000	C80	A	D	B	C	0.0001
All experiments		A	C	B	C	0.0001

Note: Letters indicate ranking (A > B > C) from Newman–Keuls procedure. Shading indicates size fraction that resulted in lowest clearance rates. ns, not significant.

Fig. 6. Percentage of carbon ingested as microzooplankton versus phytoplankton for each experiment. Shaded bars, microzooplankton; solid bars, phytoplankton.



mension dominated this fraction. Even though microzooplankton biomass was much lower than phytoplankton biomass at all stations, clearance rates of microzooplankton carbon were always higher. In the present study, microzooplankton were, in some cases, completely depleted in the experimental containers. While this depletion was not anticipated or planned, it supports our conclusions that microzooplankton are preferred over phytoplankton as prey.

The feeding preferences for larger (30–50 μm ESD) microzooplankton prey that we observed may stem from an increase in the copepod's ability to detect larger, more motile prey at greater distances than similar-sized, non-motile prey (e.g., Williamson and Vanderploeg 1988). Numerical models and visual observations indicate that particles entrained in the copepod feeding current produce a disturbance downstream that can be detected by copepod sensory structures (Bundy and Vanderploeg 2002; Visser and Jonsson 2000), and *L. sicilis* is capable of detecting large spherical prey when they are more than a copepod body length away (Bundy et al. 1998). The preferences for larger phytoplankton cells (in this case, mostly elongated diatoms and flagellates) and microzooplankton may therefore arise because motile prey and larger elongated cells produce a larger hydromechanical disturbance than small, non-motile cells (Vanderploeg et al. 1984). The chemosensory ability of *L. sicilis*, in terms of detecting phytoplankton cells that are remotely located, has not been well described. However, chemosensory-mediated selection that occurs after a particle contacts the mouthparts (i.e., taste) does play a role in controlling feeding preferences of this copepod.

Top-down control of microzooplankton

When coupled with data on ambient microzooplankton biomass, our results suggest that copepods have the potential to control microzooplankton populations in Lake Michigan during late winter – early spring. With an initial microzooplankton biomass ranging from 2.0 to 9.1 μg $C \cdot L^{-1}$ and an abundance of approximately 3 adult diaptomid copepods$\cdot L^{-1}$

during the late winter – early spring cruises (Agy 2001), copepod ingestion of microzooplankton could potentially remove between 1% and 6% of the standing crop of microzooplankton at the lowest ingestion rates observed in these experiments (0.04 μg C·copepod^{-1}·day^{-1}). At the highest ingestion rates observed (0.15 μg C·copepod^{-1}·day^{-1}), copepods could potentially remove between 5% and 23% of microzooplankton standing stock per day. These ingestion rates are conservative because they are calculated using time-averaged, rather than initial (i.e., ambient), prey concentrations in the experimental containers, and they are within the ranges observed for other small copepods in marine and estuarine systems (e.g., Gifford and Dagg 1988; Tiselius 1989). Assuming that microzooplankton production during the spring ranges from 1 to 5 μg C·L^{-1} (Carrick et al. 1992; Kovalcik 2001), adult diaptomids could potentially remove as much as 45% of new microzooplankton production per day. These conclusions are supported by studies in other freshwater systems showing that selective feeding by calanoid copepods can significantly alter microzooplankton biomass and community structure (e.g., Wiackowski et al. 1994; Jack and Gilbert 1997; Adrian and Schneider-Olt 1999).

The above estimates of copepod predation pressure on microzooplankton populations do not consider the grazing impact of diaptomid juveniles and nauplii. For example, Burns and Gilbert (1993) found that clearance rates of diaptomid juveniles feeding on microzooplankton were approximately 35% of adult clearance rates, and that, in contrast with adults, clearance rates were higher for copepods feeding on smaller (<10 μm in the longest dimension) ciliates than for larger (>16 μm) ciliates. Because of their high abundance, clearance rates of ciliates by diaptomid juveniles can equal adult clearance rates (Adrian and Schneider-Olt 1999). Abundances of diaptomid nauplii and copepodites in spring in Lake Michigan are on the order of 5 and 2.5·L^{-1}, respectively (Torke 1975; Barbiero et al. 2001), so an estimate of the grazing pressure of the entire calanoid community, including juveniles, could be in the range of 2%–43% of the standing stock and they could potentially remove as much as 80% of new microzooplankton production. It is important to note that with the inclusion of nauplii and copepodites, a wide size range of microzooplankton would be impacted.

Contribution of microbial food web to copepod production

In most freshwater and marine pelagic systems, the major consumers of picoplankton are heterotrophic flagellates and protozoans (e.g., Porter et al. 1979; Pace 1996; Calbet et al. 2001), which can, on average, account for 35% of the carbon represented by heterotrophic microorganisms in Lake Michigan in spring (Fahnenstiel et al. 1998). The preferential feeding by copepods on microzooplankton seen in this and other studies indicates that copepod grazing on protozoans can provide a mechanism for transport of the carbon found in smaller size fractions to higher trophic levels (e.g., Sherr et al. 1986; Hartmann et al. 1993). The mean clearance rates that we observed of 22 mL·copepod^{-1}·day^{-1} for copepods feeding on microzooplankton in natural seston are similar to those seen in other lakes under warmer conditions (e.g., Burns and Gilbert 1993; Jack and Gilbert 1997). Clearance rates of microzooplankton by the calanoid *Eudiaptomus gracilis* in late spring (temperature range 8.5–15.1 °C) can be 5.6 times higher than clearance rates of Chl *a* (Adrian and Schneider-Olt 1999). In the present study, the average ratio of clearance rates of copepods feeding on microzooplankton versus phytoplankton for any size fraction of Chl *a* was approximately 6.5, and our data show that, even when similarly sized phytoplankton are abundant, microzooplankton are preferred prey.

The majority of the large ciliates (e.g., oligotrichs and tintinnids) consumed by *L. sicilis* in these experiments were herbivorous or omnivorous, and therefore may have repackaged primary production, as well as bacterial production. Therefore, the picoplankton–protozoan–copepod coupling in Lake Michigan postulated by Carrick et al. (1991) is supported by the present study, where between 22% and 74% of the carbon ingested by copepods is microzooplankton carbon. Terrestrial inputs of dissolved organic carbon and phosphorus (e.g., from riverine and groundwater inputs and from resuspension events) may stimulate bacterial production (Cotner et al. 2000; Biddanda and Cotner 2002) and alter phytoplankton and microzooplankton species composition, particularly in shallow (<20 m) regions of the lake (Fahnenstiel et al. 2001; Julius and Goad 2001). Our experiments support the possibility that an increase in bacterial productivity and cell size during resuspension events, as seen during the EEGLE study (Biddanda and Cotner 2002) provides a potential carbon source to planktonic grazers through the microzooplankton–copepod link.

Preferential feeding on microzooplankton may reflect nutritional requirements of the copepod, because feeding on ciliates and heterotrophic dinoflagellates when nutritious phytoplankton are not available has been shown to increase copepod secondary production (Kleppel 1993; Kiørboe and Nielsen 1994; Ederington et al. 1995). Assuming a body weight of 6.6 μg C·copepod^{-1} (H.A. Vanderploeg, unpublished data), a specific growth rate of 0.03·day^{-1}, and an estimated growth efficiency of 0.3 for freshwater copepods at low temperatures (Hansen and Christoffersen 1995), *L. sicilis* females require 0.66 μg C·copepod^{-1}·day^{-1}, or 10% of body carbon·day^{-1} to support growth. Because calanoids reach a terminal molt at the adult stage, the majority of female growth is expressed as reproduction. In this study, copepods obtained 0.6%–2.2% and 0.6%–4.2% of body carbon·day^{-1} from microzooplankton and phytoplankton, respectively, with the carbon intake from both sources combined ranging from 2.0%–6.3% of body carbon·day^{-1} (mean, 4.1%). These estimates are based on an estimated C : Chl *a* ratio of 20, calculated empirically from cell counts and cell volumes. If we were to use a C : Chl *a* ratio of 50 as a conservative estimate, mean total carbon intake would increase to 0.5 μg C·copepod^{-1}·day^{-1}, or 7.1% of body C·day^{-1}. In only one experiment (12 March 2000 at station NB20) was intake of phytoplankton biomass sufficient to support estimated copepod reproduction. Therefore we conclude that, in spite of an abundance of phytoplankton carbon, copepods were potentially food-limited during these experiments, microzooplankton carbon contributed significantly to carbon demand, and an additional supplement from lipid reserves (Vanderploeg et al. 1992*b*) would have been required to support reproduction.

In conclusion, our results show that microzooplankton are a significant resource for copepods in Lake Michigan before and during the spring bloom in Lake Michigan. The capacity to utilize both microzooplankton and phytoplankton as important carbon sources allows *L. sicilis* to take advantage of episodic increases in heterotrophic production at a time in the year when competitors and predators are in low abundance and phytoplankton assemblages may be limited by light, temperature, or nutrients. Recent modeling efforts support this conclusion (Chen et al. 2002; Ji et al. 2002) and indicate that our findings may be applicable to other systems in which periodic physical mixing events replenish depleted nutrients, stimulate heterotrophic productivity, and lengthen the food chain to include copepods and other mesozooplankton, which in turn become links to upper trophic levels.

Acknowledgements

We thank Debra Hersha for assistance with feeding experiments and with microscopic enumeration of picoplankton. The crew and engineers of the R/V *Lake Guardian* and R/V *Laurentian* facilitated plankton collections and made it possible to conduct experiments under challenging physical conditions. We also thank Steve Ruberg, Tom Johengen, Megan Agy, and Joann Cavaletto for their help with data collection and analyses, and Dr. G.-A. Paffenhöfer for helpful comments on the manuscript.

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