

How important is bacterial carbon to planktonic grazers in a turbid, subtropical lake?

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This study examined the relative contributions of bacterial and phytoplankton production to the pelagic carbon flow of Lake Okeechobee, a large and shallow subtropical lake. Due to the predominance of cyanobacteria in this lake, we hypothesized that bacterial carbon flow would be larger than phytoplankton carbon flow to grazers. Using epifluorescent and light microscopy and radiotracer techniques, we measured the carbon biomass of planktonic functional groups and carbon flow between these groups. The functional groups that we used in this study included: picophytoplankton, autotrophic nanoflagellates (ANAN), microphytoplankton, bacteria, heterotrophic nanoflagellates (HNAN), ciliates, microzooplankton (rotifers and copepod nauplii) and macrozooplankton (cladocerans, copepodites and adult copepods). Microphytoplankton dominated the carbon biomass of all plankton, whereas the calanoid copepod, Diaptomus, dominated the carbon biomass of the grazers. Phytoplankton carbon flow often was higher than bacterial carbon flow to grazers; however, bacterial carbon constituted a large percentage of the total carbon flow to grazers ($33.7 \pm 22.4\%$). Bacterial carbon provided roughly one quarter of the carbon flow to macrozooplankton ($27.1 \pm 25.4\%$), whereas it provided half of the carbon flow to microzooplankton ($57.4 \pm 20.3\%$) and to protozoans ($47.2 \pm 25.8\%$). These results suggest that microbial pathways play an important role in the energetics of subtropical lake plankton communities. Although microbial loop pathways are important in many systems, direct bacterial carbon flow to macrozooplankton also may be important in copepod- and cyanobacteria-dominated lakes.

INTRODUCTION

Phytoplankton often is considered as the major source of food for zooplankton grazers, with bacteria serving only as a food supplement (Pomeroy, 1974; Azam *et al.*, 1983). However, in certain ecosystems bacteria may have an important role in carbon and energy flow. Bacteria can reincorporate up to 50% of the carbon that is released by phytoplankton (Pomeroy, 1974; Azam *et al.*, 1983), and they also may take up carbon that is unavailable to phytoplankton, such as recalcitrant allochthonous dissolved organic carbon (DOC) (Tranvik, 1992). This recalcitrant DOC represents additional carbon that is available for transfer to higher trophic levels.

Although cladoceran zooplankton can graze bacteria directly (Kankaala, 1988; Brendelberger, 1991), copepods are thought to graze bacteria inefficiently if at all

(Sanders *et al.*, 1989; Pace *et al.*, 1990). Protozoa can act as the link between bacteria and copepods by repackaging small bacterial particles into larger particles that copepods can more readily consume (Sherr and Sherr, 1984; Porter *et al.*, 1985). This energy transfer is less efficient than direct phytoplankton grazing pathways because of the greater number of links in the food chain between bacteria and copepods than between phytoplankton and zooplankton (Sherr and Sherr, 1984; Tranvik, 1992; Gaedke *et al.*, 1996).

Hillbricht-Ilkowska (Hillbricht-Ilkowska, 1977) proposed that the efficiency of carbon transfer from producers to zooplankton depends upon the size difference between phytoplankton and grazers. In the classic case of a north temperate lake dominated by relatively small phytoplankton (<20 μm in maximum dimension) and

large grazers (e.g. *Daphnia*), carbon transfer to grazers should be high because the large grazers can consume the small phytoplankton easily. However, in lakes that are dominated by large phytoplankton (>20 mm maximum dimension) and small grazers, carbon transfer may be low due to an inability of the grazers to consume the phytoplankton. This overlap in size between phytoplankton and zooplankton, a condition that is typical of tropical and subtropical lakes (Lewis, 1978; Fernando, 1994; Magadza, 1994), may decrease grazing on phytoplankton and cause bacteria to be an important source of carbon for zooplankton grazers.

Contemporary theory regarding the role of the bacteria in pelagic food webs is based almost exclusively on research done in the marine environment and in temperate lakes. Studies of pelagic carbon dynamics in tropical and subtropical ecosystems are rare and generally have not included bacterial-based pathways (Havens and East, 1997). This study and a companion study (Havens *et al.*, 2000) compare carbon transfer of bacterial and primary production to higher trophic levels in Lake Okeechobee, a large shallow lake in subtropical south Florida, USA. The phytoplankton in the lake is dominated by large cyanobacteria and the zooplankton is dominated by rotifers and copepods, with few small cladocerans. We tested whether bacterial carbon flow dominated the carbon flow to zooplankton grazers in Lake Okeechobee.

METHOD

Study site

Lake Okeechobee, Florida, USA (26°58' N, 80°50' W), has a large surface area (1730 km²), but a mean depth of only 2.7 m. The lake is bordered on the western edge by extensive marsh with emergent and submerged vegetation, relatively clear water, and a low nutrient content [mean total dissolved and suspended phosphorus (TP) = 0.007 mg P L⁻¹, mean total dissolved and suspended nitrogen (TN) = 1.3 mg N L⁻¹]. The lake's pelagic zone, in contrast, has high nutrient concentrations (mean TP = 0.08 mg L⁻¹, mean TN = 1.6 mg L⁻¹) and suspended solid concentrations because it is underlain by unconsolidated mud sediments that are easily stirred by wind (Havens *et al.*, 1996). Secchi depths generally are below 0.5 m and the phytoplankton biomass is much lower than in the marsh and dominated by cyanobacteria, particularly *Oscillatoria* sp. and *Lyngbya* sp. (Phlips *et al.*, 1997). Between the pelagic zone and the marsh, there is a 'nearshore' zone that at times supports large populations of macrophytes (Zimba, 1995) and possesses intermediate water transparency and phytoplankton biomass (Phlips *et al.*, 1997).

Conceptual model

To create an accurate yet manageable food web model, we used standard operationally defined categories (Sieburth *et al.*, 1978) to capture the major functional groups and pathways. Autotrophs were grouped into three categories: picoplankton, autotrophic nanoflagellates (ANAN) and microphytoplankton (nonflagellate primary producers >20 µm). We grouped heterotrophs into five categories: bacteria, heterotrophic nanoflagellates (HNAN), ciliates, microzooplankton (rotifers and copepod nauplii) and macrozooplankton (cladocerans, copepodites and adult copepods). Although both ANAN and HNAN can graze bacteria (Bird and Kalf, 1986), the relative grazing rates (GR) may differ (Porter, 1988), so we evaluated them separately.

We evaluated this model and the relative importance of the bacteria in the food web with comparisons of the magnitudes of carbon flow rates within the planktonic assemblage. Carbon flow to most protozoa originates from picoplankton (bacteria and picocyanobacteria), but micro- and macrozooplankton can graze a wider size range of particles. Therefore, we examined the carbon flow data from two major perspectives: from the bottom or the fate of bacterial and phytoplankton carbon in the food web, and from the top or the origin of carbon grazed by zooplankton. Both perspectives are important for the evaluation of the relative importance of the bacteria in the food web, but the second addresses the efficiency with which carbon is transferred to higher trophic levels.

Limnological measurements and collections

To encompass a range of conditions for hypothesis testing, we repeated collections and measurements on five occasions (July and September, 1998, and February, May, and July, 1999) at each of two sites in the lake (Fig. 1). The pelagic site (~4 m deep) was located in an area that had high light attenuation due to the resuspension of the unconsolidated mud sediments. The nearshore site (NS) (~2 m deep) was located in an unvegetated area that had sand sediments and lower light attenuation.

At each site, we measured water transparency with a Secchi disk and made profiles of photosynthetically active radiation (PAR) with a Li-Cor spherical quantum sensor and datalogger. We measured temperature, dissolved oxygen, pH and specific conductance with a Hydrolab surveyor III water quality analyzer. Using American Public Health Association (APHA, 1995) methods, we also measured chlorophyll a, total dissolved and suspended phosphorus (TP), orthophosphate (OPO₄³⁻), total dissolved and suspended Kjeldahl nitrogen (TKN), total suspended solids (TSS), dissolved inorganic carbon (DIC) and DOC. We collected samples for the water

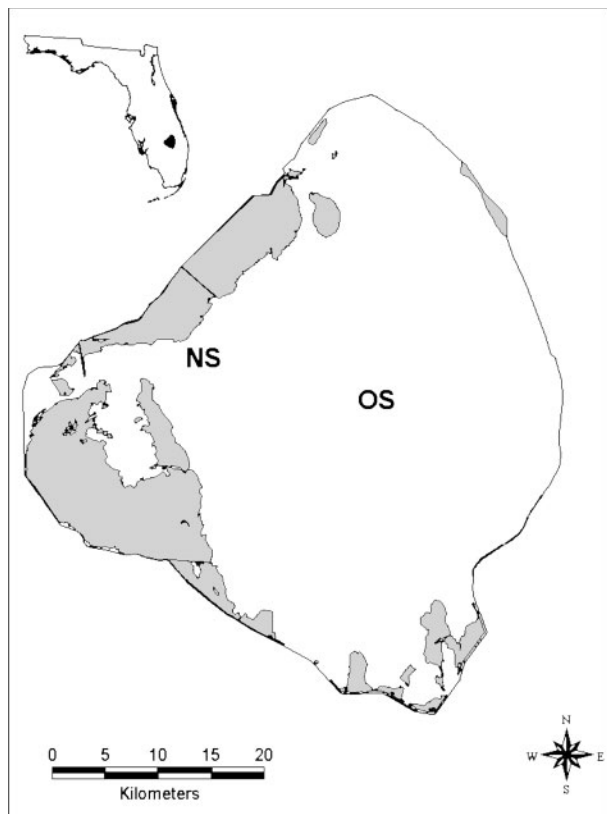


Fig. 1. Map of Lake Okeechobee. NS, nearshore site; OS, offshore site.

chemistry with a 3 cm diameter polyvinyl chloride (PVC) tube, taking integrated water column samples from ~0.5 m above the sediments to the water surface. At each site, we released the contents of several such tubes into two 20 L Nalgene carboys and removed the water chemistry samples from these carboys.

We removed concurrent samples for the measurement of plankton biomass and taxonomic composition from the same carboys. We removed and preserved in plastic (Whirl-pak Nasco®) bags two 100 mL samples each for bacteria, phytoplankton and protozoa enumeration. We filtered the remaining contents (12–20 L) of the carboys through a 41 μm nylon screen to collect microzooplankton. We collected macrozooplankton with three vertical tows of a 153 μm plankton net. We preserved bacteria samples with 0.2 μm filtered formalin, phytoplankton samples with Lugol's solution, heterotrophic protozoa samples with cacodylate-buffered glutaraldehyde and zooplankton samples with 4% sucrose formalin.

Carbon biomass measurements

For all enumerations, we counted at least 400 organisms to achieve a counting accuracy of 90% (Lund *et al.*, 1958).

Prior to enumeration, we stained bacteria with acriflavine. Both bacteria and autofluorescing picophytoplankton were enumerated with epifluorescence microscopy ($\times 1000$) after filtration onto 0.2 μm black Nucleopore® filters (Bergstrom *et al.*, 1986). We calculated biomass of bacteria and picophytoplankton from volumes measured from projected photographic slides that were calibrated with 1 μm fluorescent microspheres (Polysciences). We used the conversion factor of Scavia and Laird (Scavia and Laird, 1987) to convert volume to carbon content ($0.154 \text{ pg C } \mu\text{m}^{-3}$).

We enumerated nanoplankton with the epifluorescent method of Caron (Caron, 1983) that distinguishes between autotrophic and heterotrophic species after filtration onto 0.8 μm black Nucleopore filters and staining with primulin. We counted microphytoplankton ($>20 \mu\text{m}$ for maximum dimension) as algal units using the inverted microscope technique (Lund *et al.*, 1958). We counted algal units (individual cells, filaments or colonies) due to the predominance of filamentous and colonial cyanobacteria, for which individual cells are difficult to count accurately. We calculated biovolumes from measurements of length and width of cells that were used to approximate regular geometric solids (Wetzel and Likens, 1991). We converted nano- and microphytoplankton biovolume to carbon content using the following conversion factors: 16% of wet weight for chlorophytes, 22% of wet weight for cyanophytes and 11% of wet weight for all other algae (Lundgren, 1978; Ahlgren, 1983; Rocha and Duncan, 1985).

We counted ciliates and microzooplankton in a Sedgewick-Rafter counting cell at $\times 200$. We entered measurements of rotifers and copepod nauplii into species-specific equations given in McCauley (McCauley, 1984) for conversion into volume for rotifers and into dry weight for nauplii. To convert rotifer volumes to wet weights, we assumed unit density (1 g cm^{-3}); rotifer wet weights were converted to dry weight by assuming that dry weight = $0.1 \times$ wet weight (Pace and Orcutt, 1981). We counted macrozooplankton in a plankton counting wheel at $\times 25$. We entered body lengths of macrozooplankton into length–weight regressions given in Culver *et al.* (Culver *et al.*, 1985) to give dry weights. We converted dry weights of all zooplankton to carbon content with a conversion factor of 0.48 (Andersen and Hessen, 1991).

Carbon flow measurements

We measured net bacterial productivity using the ^3H -thymidine uptake procedure (Fuhrman and Azam, 1982) using five 5 mL samples (four replicates and one formalin-killed control) of whole lake water incubated for 1 h at ambient lake temperature. We determined the saturating concentration of ^3H -thymidine (10 nM) before

the study began with measurements of productivity at varying concentrations of ³H-thymidine. We measured the conversion factor for the number of cells produced per mole of thymidine taken up with a concurrent 24-h incubation of eight radioactive and cold samples. We preserved one pair of samples at 8-h intervals until the end of the incubation, at which time the number of cells accumulated in the cold samples was divided by the radioactivity of the corresponding hot samples. We filtered all samples onto 0.2 μm Nucleopore filters and measured radioactivity with liquid scintillation.

We measured net primary productivity as DIC uptake with the radiometric, photosynthesis-irradiance (P-E) procedure (Fahnenstiel and Scavia, 1987). On each sampling date, we incubated 18 3-mL samples of whole lake water from each site with ¹⁴C-bicarbonate in a photosynthetron. We conducted the incubation for 1 h at ambient lake temperature with one sample each at 18 irradiances (0–940 μmole photons m⁻² s⁻¹). At the end of the incubation, we filtered samples onto 0.2 μm Nucleopore filters, which we fumed over concentrated HCl for 24 h to remove unincorporated ¹⁴C-bicarbonate. We calculated photosynthesis at each irradiance level with equations given in Wetzel and Likens (Wetzel and Likens, 1991). We calculated the integrated rate of photosynthesis, P_I (μg C L⁻¹ h⁻¹) from the P-E curves generated from the incubation and the irradiance profile at each site using the equation from Jassby and Platt (Jassby and Platt, 1976).

We measured carbon flow from bacteria and phytoplankton to grazers *in situ* with a 4-L Haney chamber using previously prepared fluorescently labeled bacteria (FLB), radiolabeled bacteria and radiolabeled phytoplankton. We used bacteria both to measure bacterial carbon flow and to estimate picophytoplankton carbon flow to grazers. We isolated bacteria from the lake and cultured them in phosphate-free M9 medium to reduce their size to a volume comparable to lake bacteria after Hwang and Heath (Hwang and Heath, 1999). Final volume of cultured bacteria was slightly smaller than the lake bacteria and 27 ± 16% of the cells were clumped (Work and Havens, 2003). To prepare them for the grazing measurements, we incubated the bacteria in either 5-[4,6-(dichlorotriazin-2-yl)-amino-fluorescein (DTAF) for 2 h to label them fluorescently (Sherr *et al.*, 1987) or in ³H-thymidine for 48 h to label them radioactively.

We used two model phytoplankton, *Chlamydomonas* sp. (Carolina Biological) and *Synedra vaucheriae* (isolated from the lake), to measure carbon flow from larger organisms. We used *Chlamydomonas* (~3 μm in length, ~25 μm³ cell⁻¹ in volume) to measure autotrophic nanoflagellate carbon flow to grazers and to estimate heterotrophic nanoflagellate carbon flow to grazers. We used *Synedra* (~25 μm in length,

~100 μm³ cell⁻¹ in volume) to measure microphytoplankton carbon flow to grazers. We did not estimate carbon flow from ciliates. We incubated the two phytoplankton species separately in ¹⁴C-bicarbonate for 48 h. After the incubation, we washed all labeled organisms 3 times by centrifugation and resuspension and once more on the morning of sampling. The final concentration of the model organisms in the Haney chamber was ~20% of the natural cell density of bacteria, nanoflagellates and microphytoplankton, respectively. This proportion is substantially higher than a ‘tracer amount’, but it was necessary to obtain counts for the grazers that were significantly higher than background.

At each site, we deployed the Haney chamber nine times with three replicate incubations with each of the three model organisms. For each incubation, we lowered the chamber to 0.5 m and released the appropriate model organism for a 5-min incubation after Haney (Haney, 1971). After the incubation, we collected the contents of each chamber on sequential 200 and 40 μm Nitex screens, in order to capture macro- and microzooplankton grazers. In the measurements where grazers fed on radiolabeled particles, we washed the animals from the Nitex screens onto 20 μm filters. We also collected a 10-mL sample of the feeding suspension and filtered it onto a 0.2 μm filter. Filters were immediately placed into scintillation vials and their radioactivity was measured upon return to the laboratory (~2–4 h after collection). We calculated filtering rates (FR) for micro- and macrozooplankton, which we then converted to GR and then to carbon flow as follows:

$$FR (\text{mL L}^{-1} \text{h}^{-1}) = \frac{\left(\frac{\text{dpm of grazers}}{4\text{L}} \right)}{\left[\left(\frac{\text{dpm of feeding suspension}}{5\text{mL}} \right) \times \text{grazing time} \right]}, \quad (1)$$

where dpm = disintegrations per min

$$GR (\text{bacteria or phytoplankton L}^{-1} \text{h}^{-1}) = FR \times \text{algal or bacterial density} \quad (2)$$

$$\begin{aligned} \text{Carbon flow } (\mu\text{g C L}^{-1} \text{h}^{-1}) = & \\ & GR \times \text{bacterial or algal volume} \\ & \times \text{bacterial algal volumetric carbon content} \quad (3) \end{aligned}$$

Because some macrozooplankton may graze selectively on microphytoplankton, inclusion of all microphytoplankton in equation (2) might inflate the

carbon flow. Therefore, we determined the species of phytoplankton grazed by macrozooplankton with microscopic gut content analysis; these results were presented in a previous paper (Work and Havens, 2003). Despite the possibility of bias due to differential digestion rates of phytoplankton taxa, only the numbers and biomasses of microphytoplankton species that were observed in guts were included in the equations to calculate carbon flow.

In the case where grazers fed on fluorescent bacteria, we washed the micro- and macrozooplankton from the Nitex screens into separate plastic (Whirl-pak) bags and preserved them with 4% sucrose formalin. We examined all zooplankton collected with epifluorescence microscopy for the presence of bacteria in their guts and we presented the results of this analysis in Work and Havens (Work and Havens, 2003). At each site, we also incubated five samples of the filtrate containing fluorescent bacteria inside separate bags in a darkened cooler to allow the protozoa to continue to feed beyond the 5-min zooplankton incubation time. At 5, 10, 20, 40, 60 min, we preserved the contents of one bag with cacodylate-buffered glutaraldehyde. We quantitatively measured the ingestion of bacteria by nanoflagellates with counts of the number of fluorescing bacteria in the food vacuoles at each time interval. Ciliate food vacuoles fill quickly, so we only counted fluorescing bacteria in ciliates at 5 and 10 min. We calculated the ingestion rate (IR) as the slope of the linear regression representing ingestion (bacterial cells/protozoan) versus time. We used this IR in the following equations to calculate GR and carbon flow for protozoans:

$$\text{FR (mL L}^{-1} \text{ h}^{-1}) = \frac{\text{IR} \times \text{nanoflagellate or ciliate density}}{\text{FLB density}} \quad (4)$$

$$\text{GR (bacteria L}^{-1} \text{ h}^{-1}) = \text{FR} \times \text{natural bacterial density} \quad (5)$$

$$\text{Carbon flow (}\mu\text{g C L}^{-1} \text{ h}^{-1}) = \text{GR} \times \text{bacterial volume} \times \text{bacterial carbon content} \quad (6)$$

Data analysis

We calculated phytoplankton carbon flow as the sum of carbon flow from autotrophic picoplankton, ANAN and microphytoplankton to consumers. We calculated bacterial carbon flow as the sum of all carbon flow from

bacteria to consumers. We calculated grazing efficiency by dividing carbon flow from either bacteria or phytoplankton to their consumers by the bacterial or primary productivity, respectively. We assessed differences in carbon pools, flow rates and grazing efficiencies between stations with the Kruskal–Wallis test. We compared total carbon flow from bacteria to total carbon flow from phytoplankton for all dates/sites with a Mann–Whitney test. We examined relationships between selected variables, including food web attributes and water chemistry, with Spearman rank correlation.

We assessed the robustness of the carbon flow calculations with a sensitivity analysis. For the bacterial carbon flow rates, we substituted the bacterial carbon content conversion factor that we had chosen with other conversion factors available in the literature (Watson *et al.*, 1977; Bjørnson, 1986; Laird and Scavia, 1990; Nagata and Watanabe, 1990; Theil-Nielsen and Søndergaard, 1998). For phytoplankton carbon flow rates, we tested a wider variety of decisions: (i) we substituted carbon conversion equations available in the literature (Strathman, 1967; Verity *et al.*, 1992; Menden-Deuer and Lessard, 2000) for the conversion factors used in the bacterial content calculations; (ii) we calculated microphytoplankton carbon flow rates using *Chlamydomonas* as a model rather than *Synedra*; (iii) we eliminated the corrections for gut content analysis. We calculated 95% confidence intervals for bacterial and phytoplankton carbon flow rates and for the calculation of the percentage of the total carbon flow that originated from bacteria.

RESULTS

Temporal and spatial patterns

The two sites sampled, nearshore and offshore, differed significantly in depth, TKN and DOC ($P = 0.002$, 0.014 and 0.049 , respectively), with higher values at the offshore site (OS) (Table I). Only temperature and dissolved oxygen concentration varied seasonally ($P < .003$) with the highest values occurring in summer and winter, respectively. No other physical or chemical parameters exhibited consistent spatial or temporal patterns.

Bacterial carbon was most abundant in the summer 1998 samples (Fig. 2a and b) due to both greater abundance and larger size of the cells in these samples ($P < 0.0001$). Bacterial carbon also was slightly higher in the nearshore samples ($P = 0.02$). Although the carbon biomass differed significantly among samples for picophytoplankton, ANAN and HNAN, there were no consistent patterns (for all of the aforementioned taxa, site–date interaction term $P < 0.0001$). Microphytoplankton carbon was more abundant at the NS on all but the last date, on which

Table I: Means of limnological measurements at the two sites

Parameter	Nearshore	Offshore
Depth (m)	2.7 (2.0-3.5)	4.0 (3.5-4.5)
Secchi depth (m)	0.5 (0.35-0.65)	0.3 (0.15-0.45)
Attenuation at 1 m (%)	92.4 (79.8-98.8)	95.8 (85.5-100)
Chlorophyll a (mg m ⁻³)	48 (36-77)	22 (7-59)
Temperature (°C)	26.5 (16.1-30.8)	26.8 (17.8-30.5)
Dissolved oxygen (mg L ⁻¹)	7.2 (5.4-8.9)	6.8 (5.4-8.2)
Conductivity (µmho cm ⁻¹)	350 (330-380)	380 (350-410)
pH	8.1 (7.7-8.4)	7.9 (7.5-8.4)
Total suspended solids (mg L ⁻¹)	21.2 (14-33)	27.6 (10-73)
Total dissolved and suspended Kjeldahl nitrogen (mg L ⁻¹)	1.8 (1.4-2.2)	1.3 (1.1-1.5)
Total dissolved and suspended phosphorus (mg L ⁻¹)	0.24 (0.093-0.77)	0.12 (0.074-0.19)
OPO ₄ ⁻ (mg L ⁻¹)	0.01 (0.0005-0.05)	0.03 (0.007-0.05)
Dissolved organic carbon (mg L ⁻¹)	18 (14-24)	13 (12-14)

Ranges are presented in parentheses, N = 5.

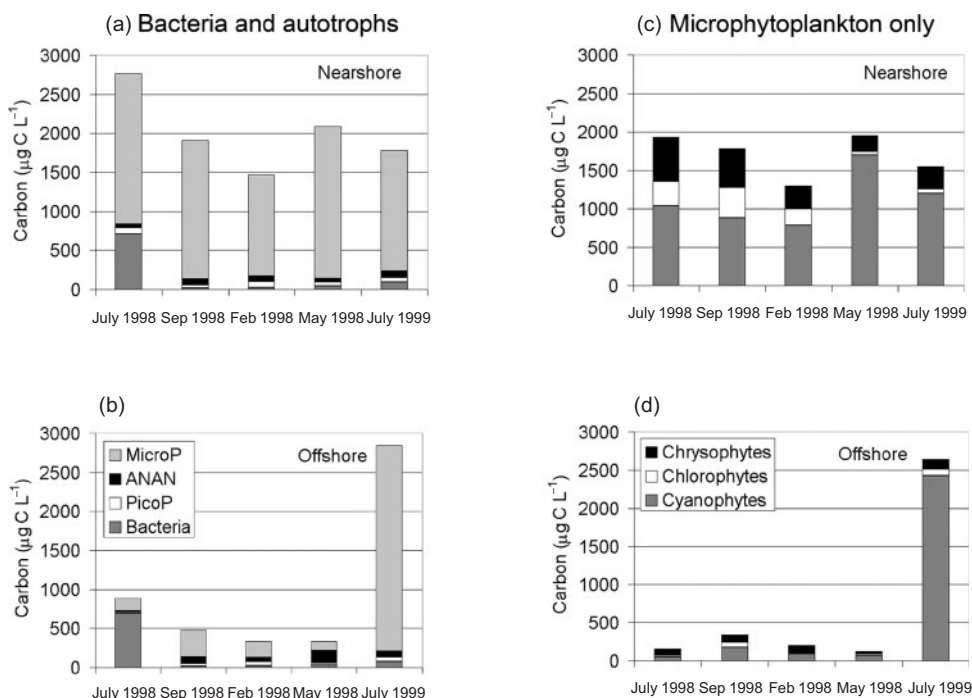


Fig. 2. Carbon biomass for bacteria and autotrophs at the nearshore (a) and offshore (b) sites and for microphytoplankton at the nearshore (c) and offshore sites (d).

both sites experienced higher biomass than on any other date (site-date interaction $P < 0.0001$). Microzooplankton carbon was more abundant at the NS ($P = 0.022$), whereas macrozooplankton carbon was more abundant at the OS in all seasons but in summer when values were similar between sites (site-date interaction $P = 0.001$).

Distribution of carbon among functional and taxonomic groups

Carbon was not distributed equally among bacteria and the primary producers ($P < 0.0001$; Fig. 2a and b). Microphytoplankton constituted the largest carbon biomass of all the plankton ($P < 0.0001$), but there were no significant

differences in carbon biomass among the bacteria and the other phytoplankton size fractions ($P > 0.8$). Within the microphytoplankton, the assemblage generally was dominated numerically by cyanobacteria ($79.3 \pm 7.3\%$), whereas total biomass was dominated by cyanobacterial carbon only at the NS: $66 \pm 15\%$, OS: $25 \pm 15\%$ (Fig. 2c and d). *Lyngbya*, *Oscillatoria* and/or *Aphanocapsa* dominated the assemblage numerically, whereas *Microcystis*, *Anabaena* or large diatoms often dominated the carbon biomass.

Despite large size differences among organisms, there were no significant differences in the carbon biomass of the heterotrophs ($P = 0.77$; Fig. 3a and b). Within the zooplankton, copepods constituted $80.7 \pm 19.1\%$ of the number and $77.8 \pm 23.3\%$ of biomass. *Diaptomus dorsalis* dominated the macrozooplankton in all samples ($64.4 \pm 17.5\%$; Fig. 3c and d). *Mesocyclops edax* and *Tropocyclops prasinus* were less abundant and constituted $16.3 \pm 12.4\%$ of the macrozooplankton. *Diaphanosoma brachyrum* was the only consistently abundant cladoceran ($10.7 \pm 12.4\%$ of the zooplankton assemblage). *Daphnia lunholzi* attained a relatively high biomass in summer 1998 samples (8.1% of total zooplankton assemblage) and *Daphnia ambigua* in the spring 1999 samples (5.4% of the total assemblage), but these biomasses were low relative to *Diaptomus*.

Bacterial and primary production

Rates of bacterial and primary production did not differ significantly from each other ($P = 0.13$; Fig. 4), although

bacterial productivity was more variable ($125.2 \pm 51.8 \text{ mg L}^{-1} \text{ h}^{-1}$) than primary productivity ($58.2 \pm 19.3 \text{ mg L}^{-1} \text{ h}^{-1}$). Bacterial productivity was significantly correlated with bacterial biomass ($r = 0.72$, $P = 0.019$), but not with primary productivity ($r = 0.30$, $P = 0.40$) or with phytoplankton biomass ($r = 0.55$, $P = 0.098$). Primary productivity was significantly correlated with chlorophyll a concentration ($r = 0.86$, $P = 0.001$), total phytoplankton biomass ($r = 0.80$, $P = 0.006$) and microphytoplankton biomass ($r = 0.80$, $P = 0.006$).

Fate of bacterial and algal carbon

Bacterial carbon contributed $33.7 \pm 22.4\%$ of the total carbon flow to grazers and, as a result, the contributions of bacterial and algal carbon to total carbon flow to grazers did not differ significantly ($P = 0.2$; Fig. 5). However, algal and bacterial carbon flow differed significantly for macrozooplankton ($P = 0.01$), where bacterial carbon made a smaller contribution to total carbon flow ($27.1 \pm 25.4\%$) than algal carbon. Bacterial carbon contributed $47.2 \pm 25.8\%$ of the total carbon flow to protozoans and $57.4 \pm 20.3\%$ of the total carbon flow to microzooplankton.

Grazers differed in the total amount of bacterial carbon that they consumed ($P < 0.0001$; Fig. 5a and b, Table III). Micro- and macrozooplankton consumed comparable amounts of bacterial carbon ($P = 0.23$), but their consumption of bacterial carbon was greater

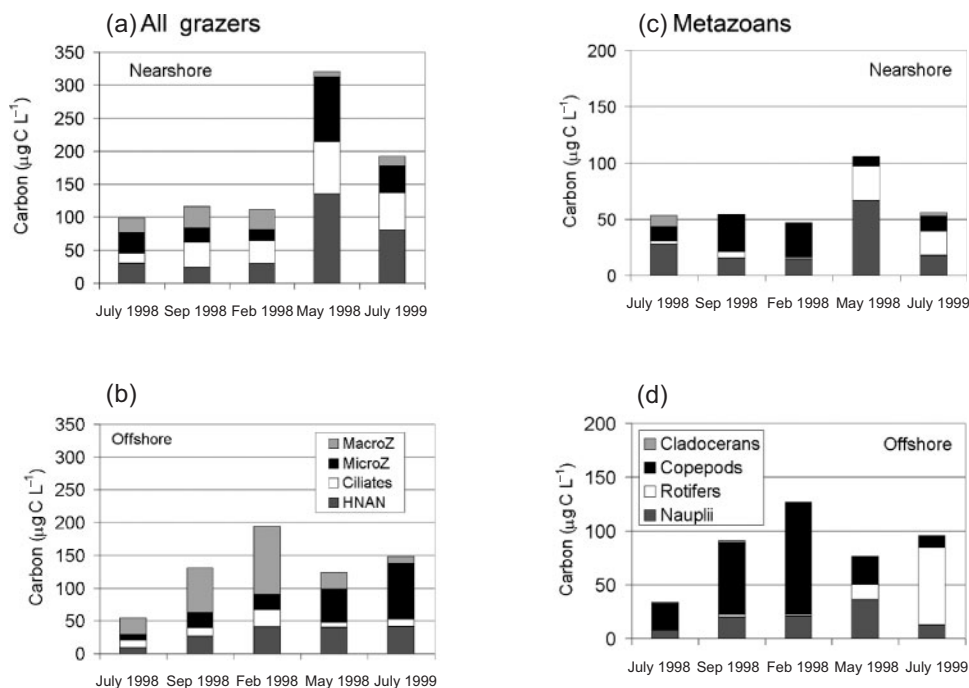


Fig. 3. Carbon biomass for all grazers at the nearshore (a) and offshore (b) sites and for zooplankton at the nearshore (c) and offshore sites (d).

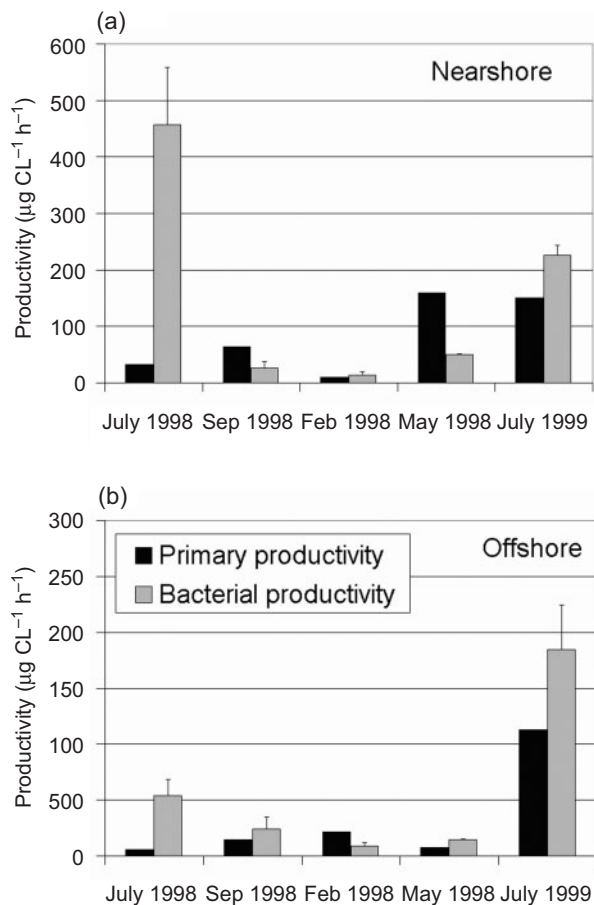


Fig. 4. Primary and bacterial productivity at the nearshore (a) and offshore (b) sites.

than that of the protozoans ($P < 0.006$). The consumption of bacterial carbon did not differ among protozoans ($P > 0.1$).

Total algal carbon consumption also differed among grazers ($P < 0.0001$; Fig. 5c and d, Table IV). The consumption of algal carbon was similar for micro- and macrozooplankton ($P = 0.18$) and greater than the consumption of algal carbon by the protozoa ($P < 0.02$). Taxonomic patterns of carbon consumption of picophytoplankton mirrored the patterns for total algal carbon consumption. Carbon flow differed between micro- and macrozooplankton only for ANAN ($P = 0.001$), where carbon flow was greater to macrozooplankton. For microphytoplankton, carbon flow was calculated only for macrozooplankton.

The efficiency with which the entire planktonic assemblage grazed bacterial and algal carbon differed ($P = 0.04$). A greater proportion of the carbon produced by phytoplankton was grazed than the proportion of carbon produced by bacteria (77.6 ± 77.1 versus $30.3 \pm 31.4\%$).

The origin of carbon grazed by zooplankton

Carbon flow differed among possible food items in the diet of zooplankton ($P = 0.001$; Fig. 6). Microzooplankton consumed more bacterial carbon than all other measured food items ($P < 0.02$; Fig. 6a and b). Bacterial carbon flow to microzooplankton was correlated significantly with bacterial biomass ($r = 0.72$, $P = 0.002$), whereas phytoplankton carbon flow to microzooplankton was not correlated significantly with either picophytoplankton or ANAN biomass ($P > 0.1$).

Gut content analysis of macrozooplankton (Work and Havens, 2003) indicated that both filaments and colonies were consumed: $34.5 \pm 19.5\%$ of all microphytoplankton consumed were filaments and $9.3 \pm 10.5\%$ were colonies. Therefore, we included a variable fraction of the cyanobacteria in the calculations of carbon flow from microphytoplankton to macrozooplankton. The resulting carbon flow from microphytoplankton to macrozooplankton was higher than from all other food items ($P < 0.03$; Fig. 6c and d). Bacterial carbon made the second largest contribution to the macrozooplankton diet, followed by picophytoplankton, and finally the nanoflagellates. Carbon flow from bacteria was correlated significantly with bacterial biomass ($r = 0.57$, $P = 0.012$). Carbon flow from phytoplankton to macrozooplankton was correlated significantly with picophytoplankton biomass ($r = 0.81$, $P = 0.005$), but not with ANAN or microphytoplankton biomass ($r = -0.25$, $P = 0.5$; $r = 0.61$, $P = 0.06$, respectively).

Sensitivity analysis

Carbon flow values varied a great deal with changes in the conversion factors or equations, model organisms and diet composition used (Table II). Phytoplankton carbon flow varied by $39.7 \pm 16.4\%$ from the mean of all carbon flow values used in the sensitivity analysis, whereas bacterial carbon flow varied by 74.0% and the proportion bacterial carbon flow of total flow varied by $87.9 \pm 41.8\%$. We adopted phytoplankton carbon flows that were or approached the maximum values calculated in the sensitivity analysis, whereas our bacterial carbon flows always underestimated the means. As a result, when we calculated the proportion of bacterial carbon flow of the total carbon flow using the adopted phytoplankton and bacterial carbon values, the proportions always approached the mean values calculated in the sensitivity analysis (27.1 ± 25.4 versus $25.4 \pm 23.8\%$, Table II).

DISCUSSION

This study was conducted to determine whether bacterial carbon was more important than phytoplankton

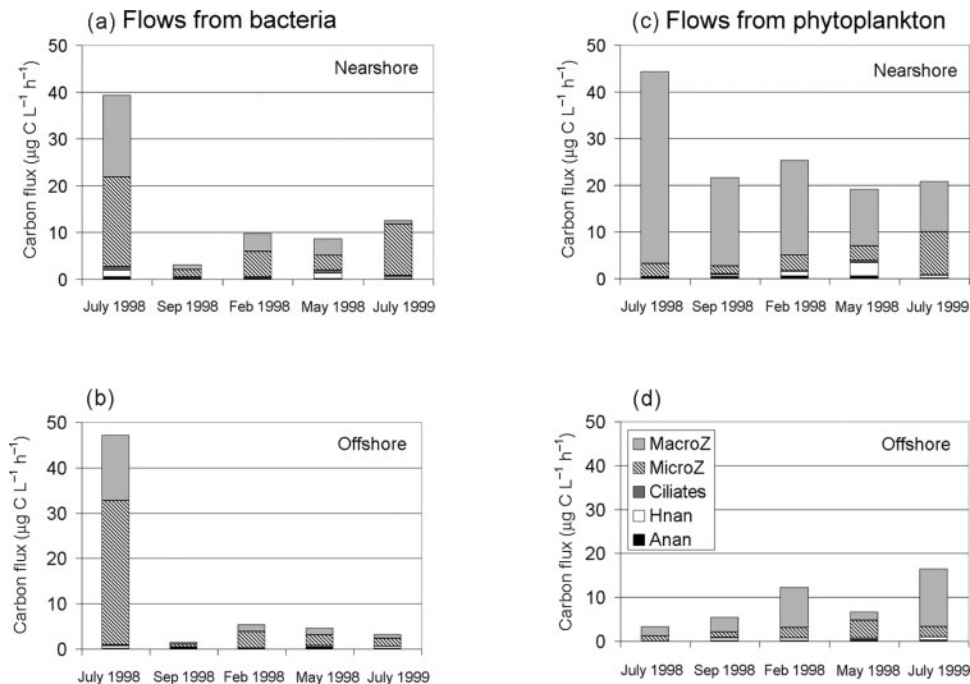


Fig. 5. Carbon flow from bacteria to grazers at the nearshore (a) and offshore (b) sites and from phytoplankton to grazers at the nearshore (c) and offshore (d) sites. The phytoplankton is represented by picophytoplankton, autotrophic nanoflagellates (ANAN) and microphytoplankton.

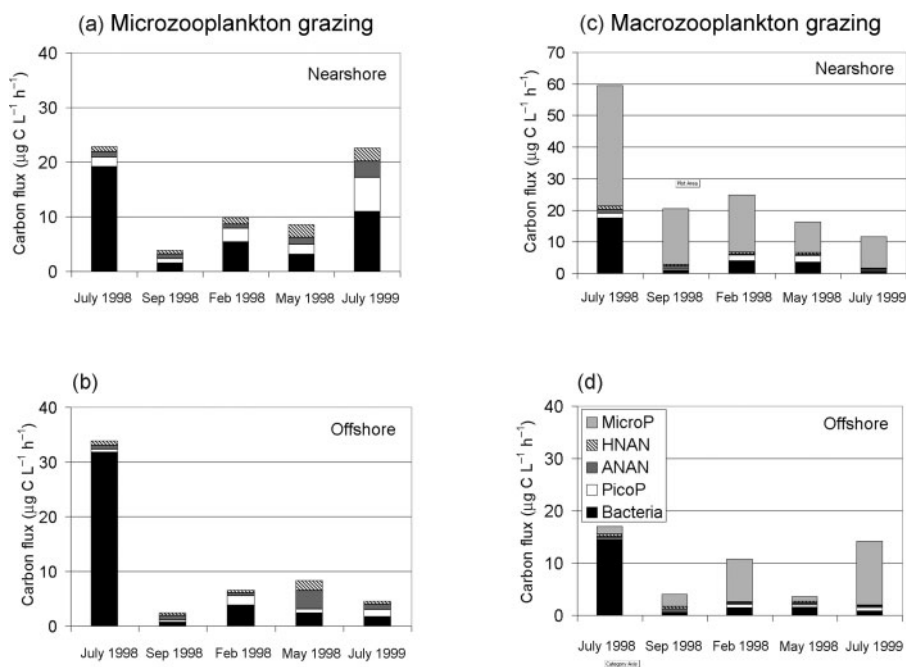


Fig. 6. Carbon flow to microzooplankton at the nearshore (a) and offshore (b) sites and to macrozooplankton at the nearshore (c) and offshore (d) sites. Heterotrophs are represented by bacteria and heterotrophic nanoflagellates (HNAN) and phytoplankton are represented by picophytoplankton (PicoP), autotrophic nanoflagellates (ANAN) and microphytoplankton (MicroP). We assumed that microzooplankton did not consume large phytoplankton, so microphytoplankton were not included in (a) and (b).

Table II: Sensitivity analysis to determine the effects of changing the carbon conversion factors and other corrections on the magnitude of carbon flow to macrozooplankton ($\mu\text{g C L}^{-1} \text{h}^{-1}$)

Variable	Date	Station	Adopted	Minimum	Maximum	Average
PCF	9 July 1998	NS	44.3	19.1	44.3	26.9
		OS	3.4	3.3	5.7	4.2
	29 September 1998	NS	23.4	12.4	23.4	15.5
		OS	5.2	4.4	6.6	5.3
	24 February 1999	NS	25.5	10.3	25.5	17.0
		OS	10.6	4.2	10.6	8.0
	19 May 1999	NS	19.0	12.2	19.0	14.5
		OS	6.7	6.7	13.1	10.3
	27 July 1999	NS	24.1	17.2	24.5	20.8
		OS	15.2	5.0	15.2	9.3
BCF	9 July 1998	NS	17.1	13.3	41.0	23.5
		OS	17.1	13.3	41.0	23.6
	29 September 1998	NS	0.65	0.51	1.6	0.90
		OS	0.36	0.28	0.87	0.50
	24 February 1999	NS	0.88	0.67	2.1	1.2
		OS	0.66	0.52	1.6	0.92
	19 May 1999	NS	2.6	2.0	6.3	3.6
		OS	1.8	1.4	4.3	2.5
	27 July 1999	NS	6.6	5.1	15.8	9.1
		OS	1.9	1.5	4.6	2.7
% BCF of total CF	9 July 1998	NS	54.6	23.1	68.2	45.9
		OS	86.2	70.0	92.6	83.4
	29 September 1998	NS	6.3	2.1	11.2	5.6
		OS	9.8	4.1	16.3	8.6
	24 February 1999	NS	7.4	2.6	17.1	7.2
		OS	9.3	4.6	27.7	11.0
	19 May 1999	NS	22.4	9.6	34.0	19.7
		OS	18.6	9.6	39.0	19.4
	27 July 1999	NS	31.5	17.3	47.9	30.0
		OS	25.1	9.0	48.2	23.7

PCF, phytoplankton carbon flow; BCF, bacterial carbon flow; %BCF to total CF, percent of the total carbon flow that was bacterial carbon flow; NS, nearshore site; OS, offshore site. The analysis evaluated a wide variety of carbon content conversion factors, equations and methodological decisions and produced a large array of values for each parameter. The results are summarized with the value adopted for the study and the minimum and maximum values produced by the perturbations of the sensitivity analysis. Additional bacterial conversion factors used are $0.12 \text{ pg } \mu\text{m}^{-3}$ (Watson *et al.*, 1977), $0.35 \text{ pg } \mu\text{m}^{-3}$ (Bjørnsen, 1986), $0.37 \text{ pg } \mu\text{m}^{-3}$ and $0.18 \text{ pg } \mu\text{m}^{-3}$ (Laird and Scavia, 1990), $0.136 \text{ pg } \mu\text{m}^{-3}$ (Nagata and Watanabe, 1990), $0.35 \text{ pg } \mu\text{m}^{-3}$ (Theil-Nielsen and Søndergaard, 1998) and $0.138 \text{ pg } \mu\text{m}^{-3}$ and $0.196 \text{ pg } \mu\text{m}^{-3}$ (Hwang and Heath, 1999). Additional algal equations used for diatoms, $\log C = -0.422 + 0.758 \times \log(\text{volume})$, for all other phytoplankton, $\log C = -0.314 + 0.712 \times \log(\text{volume})$ (Strathman, 1967); $\log C = -0.363 + 0.863 \times \log(\text{biovolume})$ (Verity *et al.*, 1992); for diatoms, $\log C = -0.665 + 0.939 \times \log(\text{volume})$, for all other phytoplankton, $\log C = -0.541 + 0.86 + \log(\text{volume})$ (Menden-Deuer and Lessard, 2000).

carbon in the plankton energetics of a highly turbid, eutrophic, subtropical lake. Bacterial production in Lake Okeechobee was high ($125.2 \pm 51.8 \text{ mg L}^{-1} \text{h}^{-1}$) and comparable to phytoplankton production ($58.2 \pm 19.3 \text{ mg L}^{-1} \text{h}^{-1}$). Although grazing efficiency was low for bacteria ($30.3 \pm 31.4\%$) and high for phytoplankton ($77.6 \pm 77.1\%$), bacterial carbon constituted half of the total carbon flow to grazers. Furthermore, bacterial

carbon provided half of the carbon flow to microzooplankton and one quarter of the carbon flow to macrozooplankton, despite the predominance of copepods in the zooplankton assemblage.

Relatively high bacterial carbon flow to a zooplankton assemblage dominated by copepods is surprising given the previous literature on copepod feeding selectivity and the role of copepods in pelagic food webs (Sanders

et al., 1989; Pace *et al.*, 1990; Burns and Schallenberg, 1996). Fenchel (Fenchel, 1988) proposed that predator:prey length ratios for plankton typically average 1:10 and that ratios greater than 1:100 are rare. Copepod : bacteria length ratios for Lake Okeechobee exceeded 1:1000, which suggests that copepods should graze larger food items (phytoplankton, protozoans, rotifers) than bacteria. In fact, studies typically have assumed and found that rotifers and cladocerans have dominated bacterivory (Sanders *et al.*, 1989; Hwang and Heath, 1999; Thouvenot *et al.*, 1999).

Our carbon flow data may be explained by Hillbricht-Ilkowska's (Hillbricht-Ilkowska, 1977) hypothesis that phytoplankton grazing should be low in lakes that are dominated by small zooplankton and large phytoplankton (e.g. *Lyngbya*). If a lake is dominated by large, filamentous and colonial cyanobacteria that are notoriously poor food for zooplankton (Infante and Abella, 1985; Threlkeld, 1985; Gliwicz, 1990; Ferrao-Filho *et al.*, 2000), bacteria might serve as an important source of carbon in the planktonic food web. Given the predominance of copepods in Lake Okeechobee, we expected that the bacterial carbon would enter the plankton food web primarily through microbial loop pathways (e.g. protozoan grazing), rather than by direct uptake by macrozooplankton. However, the contribution of protozoa to total bacterial carbon flow to grazers was low and bacterial carbon primarily entered the food web through direct uptake by micro- and macrozooplankton.

Direct uptake of bacteria by copepods could be the result of either selectivity for bacteria or incidental uptake with other cells or particles. Copepods feed highly selectively (DeMott, 1986, 1988) and they generally are considered to be either phytoplanktivorous (calanoids) or predatory on protozoans and rotifers (cyclopods) rather than bacterivorous (Williamson, 1983; DeMott, 1986; Stoecker and Capuzzo, 1990). We found that all species of zooplankton, including calanoid and cyclopoid copepods, consumed bacteria in Lake Okeechobee (Work and Havens, 2003). However, all species of zooplankton also contained phytoplankton (Work and Havens, 2003). Furthermore, the turbidity of Lake Okeechobee typically is high (Table I) and bacteria may adsorb to sediment particles, which then may be grazed by zooplankton (Gliwicz, 1986). Sediment particles with adsorbed bacteria may reduce the predator:prey length ratio so that the larger particles are more readily grazed by copepods (Fenchel, 1988). Although only the FLB were present in the water for 5 min, we cannot determine whether the copepods consumed bacteria individually, bacteria associated with phytoplankton, or bacteria associated with sediment.

Nevertheless, the consumption of bacteria by copepods occurred commonly in this study.

The relative lack of importance of protozoa in the flow of carbon in Lake Okeechobee is surprising given the abundance of literature that has suggested that protozoa are major bacteria grazers in many pelagic ecosystems (Sanders *et al.*, 1989; Pace *et al.*, 1990; Weisse, 1990). Although the densities of protozoa measured in this study were comparable to other ecosystems, the FR and GR measured for the protozoa in this study (Table III) were slightly lower than have been measured (HNAN = 0.4–6.5 nL individual⁻¹ h⁻¹, ANAN = 0.4–32 nL individual⁻¹ h⁻¹, Ciliates = 29–152 nL individual⁻¹ h⁻¹) in many other studies (Epstein and Shiaris, 1992; Hwang and Heath, 1997; Thouvenot *et al.*, 1999). The micro- and macrozooplankton FR and GR that we measured (Table III) were comparable to rates that have been measured for bacteria (microzooplankton = 0.004–6.2 mL individual⁻¹ h⁻¹ and macrozooplankton = 0.02–2.8 mL individual⁻¹ h⁻¹) in other studies (Peterson *et al.*, 1978; Porter, 1984; Kankaala, 1988; Ooms-Wilms, 1997; Tóth and Kato, 1997). Similarly, our measurements (Table IV) were comparable to rates measured for micro- and macrozooplankton grazing on phytoplankton (microzooplankton = 0.02–0.13 mL individual⁻¹ h⁻¹ and macrozooplankton = 0.10–8.3 mL individual⁻¹ h⁻¹) in other studies (Bogdan and McNaught, 1975; Starkweather and Gilbert, 1977; Janicki and DeCosta, 1984; Stemberger and Gilbert, 1987; Kankaala, 1988; Lair and Ali, 1990; Havens, 1991). Our results were similar despite the focus on rotifers and cladocerans in these published grazing studies and the predominance of copepods on our study.

In this study, protozoan and zooplankton GR were estimated with two different methods: direct uptake of fluorescent bacteria over time and uptake of radiolabel, respectively. It is possible, given our slightly low FR for protozoa, that we underestimated the contribution of protozoa to the carbon flow in Lake Okeechobee. This suggestion is further supported by a study (Havens *et al.*, 2000) that was conducted concurrently with this study, albeit using different methods. Havens *et al.* (Havens *et al.*, 2000) was a laboratory study in which radiolabel (either DOC or DIC) was added to whole water. The water was then filtered to determine the proportion of label in each size fraction (percent label transfer or PLT). In both DOC and DIC additions, the majority of the label ended up in the 20–40 µm size fraction, which suggests a strong influence of protozoans and small rotifers on carbon flow. As a result, the proportion of the label transferred to the macrozooplankton size fraction was quite low (0.1–1%). For the current study,

Table III: Ingestion, clearance, filtering, grazing and carbon flow rates for plankton feeding upon bacteria at the nearshore (NS) and offshore (OS) sites

Plankter	Date	Ingestion rate		Clearance rate		Filtering rate		Grazing rate		Carbon flux	
		NS (bacteria individual ⁻¹ h ⁻¹)	OS (bacteria individual ⁻¹ h ⁻¹)	NS (nL individual ⁻¹ h ⁻¹)	OS (nL individual ⁻¹ h ⁻¹)	NS (mL L ⁻¹ h ⁻¹)	OS (mL L ⁻¹ h ⁻¹)	NS (×10 ⁶ bacteria L ⁻¹ h ⁻¹)	OS (×10 ⁶ bacteria L ⁻¹ h ⁻¹)	NS (μg C L ⁻¹ h ⁻¹)	OS (μg C L ⁻¹ h ⁻¹)
HNAN	9 July 1998	0.74	0.55	0.15	0.11	2.09	0.90	15.8	8.66	1.47	0.61
	29 September 1998	0.91	1.33	0.65	0.95	6.16	10.3	15.0	16.7	0.12	0.17
	24 February 1999	0.45	0.47	0.32	0.34	6.13	5.61	12.0	11.5	0.14	0.12
	19 May 1999	1.08	1.33	0.97	0.63	2.72	9.20	15.7	25.3	1.19	0.32
	27 July 1999	1.97	1.03	0.35	0.43	5.84	6.61	85.3	20.8	0.56	0.48
ANAN	9 July 1998	0.12	0.066	0.024	0.013	0.71	0.14	5.44	1.32	0.51	0.093
	29 September 1998	0.41	0.12	0.29	0.086	5.83	1.60	14.2	2.58	0.11	0.026
	24 February 1999	0.28	0.048	0.20	0.034	3.70	1.52	7.25	3.11	0.083	0.034
	19 May 1999	0.21	0.64	0.19	0.30	2.70	1.24	8.47	34.0	0.12	0.42
	27 July 1999	0.19	0.26	0.034	0.11	1.00	2.18	2.68	6.87	0.10	0.16
Ciliates	9 July 1998	60.5	60.6	12.1	12.1	0.45	0.15	3.41	1.49	0.32	0.11
	29 September 1998	89.7	77.8	81.5	55.6	2.26	1.88	5.49	3.04	0.044	0.031
	24 February 1999	32.1	43.1	23.0	30.8	1.01	0.13	1.98	0.26	0.023	0.003
	19 May 1999	76.9	100.1	69.9	47.7	4.05	0.47	12.7	1.30	0.18	0.016
	27 July 1999	74.8	87.3	13.4	36.4	0.76	0.36	2.08	1.13	0.07	0.03
Micro zoo	9 July 1998			0.001	0.028	9.99	7.29	76.1	70.2	7.73	11.20
	29 September 1998			0.013	0.007	8.14	2.79	19.8	4.49	0.22	0.091
	24 February 1999			0.016	0.048	11.9	6.40	23.3	13.1	0.37	0.38
	19 May 1999			0.005	0.026	20.5	11.8	64.3	32.6	0.52	0.63
	27 July 1999			0.012	0.009	3.88	5.70	10.4	17.9	5.48	0.85
Macro zoo	9 July 1998			0.27	0.21	10.9	16.0	83.2	154	7.06	5.09
	29 September 1998			0.29	0.15	11.3	5.66	27.6	9.11	0.16	0.045
	24 February 1999			1.09	0.39	16.3	17.0	32.0	34.8	0.27	0.14
	19 May 1999			2.49	0.73	17.7	18.2	55.4	50.2	0.60	0.41
	27 July 1999			3.03	1.12	57.0	11.7	153	36.6	0.37	0.42

HNAN, heterotrophic nanoflagellates; ANAN, autotrophic nanoflagellates. Filtering rates (FR) were measured from Haney chamber experiments using fluorescently labeled bacteria (FLB) (protists) or radiolabeled phytoplankton (micro- and macrozooplankton). Protist FR were calculated as averages of all taxa examined for each functional group and zooplankton FR were calculated from composite ambient samples collected in the Haney chamber. All other rates were calculated from FR and biomass measurements.

Table IV: Ingestion, clearance, filtering, grazing and carbon flow rates for plankton feeding upon phytoplankton at the nearshore (NS) and offshore (OS) sites

Plankter	Date	Clearance rate		Filtering rate		Grazing rate		Carbon flux	
		NS	OS	NS	OS	NS	OS	NS	OS
		(mL individual ⁻¹ h ⁻¹)	(mL individual ⁻¹ h ⁻¹)	(mL L ⁻¹ h ⁻¹)	(mL L ⁻¹ h ⁻¹)	(×10 ⁶ units L ⁻¹ h ⁻¹)	(×10 ⁶ units L ⁻¹ h ⁻¹)	(μg C L ⁻¹ h ⁻¹)	(μg C L ⁻¹ h ⁻¹)
Micro zoo	9 July 1998	0.003	0.24	22.2	62.8	0.37	0.49	1.18	1.41
	29 September 1998	0.019	0.024	11.6	10.3	0.23	0.19	1.41	1.31
	24 February 1999	0.024	0.082	10.8	10.9	0.20	0.48	1.21	1.88
	19 May 1999	0.005	0.10	22.9	45.5	0.33	1.88	1.99	7.35
	27 July 1999	0.12	0.013	39.3	8.44	1.15	0.11	5.29	1.36
Macro zoo	9 July 1998	0.58	0.41	23.5	31.7	0.39	0.25	1.91	0.71
<i>w/Chlamydomonas</i>									
	29 September 1998	0.23	0.24	8.88	8.82	0.19	0.16	1.08	1.12
	24 February 1999	0.37	0.13	5.52	5.45	0.10	0.24	0.62	0.63
	19 May 1999	0.75	0.26	5.34	6.45	0.076	0.27	0.46	0.69
	27 July 1999	0.14	0.24	2.66	2.55	0.078	0.034	0.36	0.41
Macro zoo	9 July 1998	0.45	0.13	18.2	10.0	0.41	0.021	24.5	1.03
<i>w/Synedra</i>									
	29 September 1998	0.25	0.19	9.93	0.23	0.23	0.041	10.5	1.66
	24 February 1999	1.17	0.92	17.5	39.7	0.26	0.28	10.5	6.67
	19 May 1999	0.71	0.33	5.02	8.17	0.082	0.031	5.25	0.72
	27 July 1999	0.35	0.45	6.55	4.66	0.13	0.34	6.30	7.81

Filtering rates (FR) were measured from Haney chamber experiments using radiolabeled bacteria. Zooplankton FR were calculated from composite ambient samples collected in the Haney chamber. All other rates were calculated from FR and biomass measurements. Algal units (cells, colonies or filaments, respectively) were used to calculate grazing rates (GR).

if percent carbon transfer is calculated as carbon flow to macrozooplankton/carbon flow to all organisms (productivity + grazing), then the percent carbon transfer is $3.7 \pm 2.8\%$ for DOC and $23.4 \pm 18.1\%$ for DIC. These values are approximately one to two orders of magnitude higher than the values measured by the PLT method ($\sim 0.4\%$ for DOC and $\sim 0.2\%$ for DIC) used in Havens *et al.* (Havens *et al.*, 2000). An underestimate of protozoan grazing on bacteria and picophytoplankton could account for the higher percent carbon transfer to macrozooplankton calculated in this study. However, because the PLT method only measures the percent of the total activity that occurs in each size fraction, it is impossible to compare the GR of size fractions to determine the likely cause of the discrepancy between the two studies.

Grazing efficiency or percent carbon transfer per se may not adequately represent the importance of bacterial carbon in Lake Okeechobee. Although the grazing efficiency for bacteria was lower than that for phytoplankton, the total carbon flow from bacteria comprised a large proportion of the total carbon flow to macrozooplankton ($27 \pm 25\%$). This pattern was even stronger for the microzooplankton ($57 \pm 20\%$), which was composed of juvenile stages of macrozooplankton as well as rotifers. Furthermore, our phytoplankton carbon flow measurements are likely to be inflated, even with our attempt to correct for the predominance of low-quality food items, and this problem would have decreased the proportion of the total carbon flow that originated as bacteria. The microphytoplankton carbon flows to grazers were calculated from FR on a diatom, albeit a diatom cultured directly from the lake. Diatoms are of considerably higher quality food than cyanobacteria and it is likely that the GR measured for the diatom were higher than if we had been able to use a cyanobacterium as a model, particularly given our short incubation period. We were unable to use a large cyanobacterium as a model organism because both filaments and colonies can clog the screens used to capture the zooplankton and, as a result, they can artificially elevate the radioactivity of the zooplankton captured on the screens. Furthermore, although all zooplankton taxa were observed with cyanobacteria in their guts (Work and Havens, 2003), the animals used in the gut content analysis came from the lake population that had been feeding for longer than our incubation period. Cyanobacteria may be more resistant to digestion than many other taxa (de Bernardi and Giussani, 1990), so the cyanobacteria that we observed in the zooplankton guts may have accumulated over a longer period than our incubation period. Our use of their density in the FR may have further inflated the total FR on phytoplankton. However, an overestimate of grazing on microphytoplankton would

only increase the importance of bacteria as a direct source of carbon to zooplankton in Lake Okeechobee.

The approaches taken in carbon flow studies have been highly variable, making comparisons difficult. Our sensitivity analysis indicated that these types of measurements may be highly dependent on small methodological decisions, such as carbon conversion factors. Furthermore, the high variability in carbon flow between sampling dates in our subtropical lake, a type of lake which typically experiences relatively low variability in annual and diel irradiance and temperature (Talling and Lemoalle, 1998), likely will be magnified in a temperate lake. Despite these problems, the data provide some new insights about the way in which cyanobacteria- and copepod-dominated food webs may operate. First, bacteria may be an important source of carbon in eutrophic lakes dominated by cyanobacteria. Second, copepod consumption of bacteria may result in considerable direct carbon transfer from bacteria to macrozooplankton in copepod-dominated systems. Given the importance of these findings, future research should aim to quantify (using consistent methods) the utilization of bacterial production in lakes across a wide range of relative copepod versus cladoceran biomass.

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