

SHORT COMMUNICATION

Relative efficiencies of carbon transfer from bacteria and algae to zooplankton in a subtropical lake

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Abstract. Direct measurements were made of carbon transfer efficiency from bacteria and algae to zooplankton in subtropical Lake Okeechobee, Florida, USA. A radio-tracer method was used to determine carbon transfer efficiency to the >200 μm size class, comparing results from treatments where the label was added as ^{14}C glucose or ^{14}C -bicarbonate. The studies were done at a near-shore and an offshore site, and repeated on five occasions between July 1998 and 1999. The results displayed little variation with location, month, or type of tracer used. After 4 h incubations, between 0.1 and 1.0% of the particulate activity occurred in the >200 μm size class. Low efficiencies suggest a considerable loss of carbon from the copepod- and cyanobacteria-dominated food web; this seems to be typical of highly eutrophic lakes.

When phytoplankton carry out photosynthesis, a substantial amount of the fixed carbon is excreted to the surrounding water as dissolved organic carbon (DOC) (Cole *et al.*, 1982). The carbon can be taken up by bacteria, which in turn are grazed by protozoa and metazoan plankton. This pathway for carbon flow has been referred to as the microbial loop (Azam *et al.*, 1983). It can transport large quantities of carbon in freshwater lakes (Weisse and Muller, 1990) and the open ocean (Cole *et al.*, 1988) and is the route whereby allochthonous DOC enters the food web (Jansson *et al.*, 1999).

A central issue in research dealing with the microbial loop is whether bacteria-based pathways transfer carbon less efficiently than algal-based pathways (Ducklow *et al.*, 1986; Sherr and Sherr, 1987). This issue can be addressed by two different methods. The first requires measurement of carbon transfer at all known links in the grazing and microbial food webs. This has been done for a limited number of systems [e.g. (Sondergaard *et al.*, 1988; Kankaala *et al.*, 1996; Jansson *et al.*, 1999)]. The second method (Ducklow *et al.*, 1986) is to measure the efficiency of carbon transfer by adding a radio-tracer to the community and examining the transfer of activity over time into the macro-zooplankton size class. Most recently, Koshikawa *et al.* (Koshikawa *et al.*, 1996) used this method to compare efficiencies of the bacterial and algal-based pathways of an inland sea. To our knowledge, only two other studies (Wylie and Currie, 1991; Havens, 1994) have used the approach.

Here, we report the results of a radio-tracer study to compare carbon transfer efficiencies from bacteria and algae to macro-zooplankton in a large subtropical lake. In a more comprehensive study conducted at the same time (Work *et al.*, submitted), we measured the carbon biomass of all plankton components and major pathways of carbon flux. We draw on results from that study to explain patterns in ecological transfer efficiency.

Samples were collected from a near-shore and offshore location in Lake Okeechobee, located at 26°58'N 80°50'W in south Florida, USA. This lake has a surface area of over 1700 km² and a mean depth of near 3.0 m; it is highly eutrophic (Aumen, 1995).

Water temperature was measured using a Hydrolab multi-parameter analyzer and transparencies were measured with a 20 cm black and white Secchi disk. Samples for laboratory studies of carbon transfer were collected with a 5 cm diameter plastic tube that sampled the entire water column to within 0.25 m of the sediments. Samples were collected until a 20 l carboy was entirely filled, after which it was placed under a protective tarp to avoid exposure to sunlight. A second carboy was filled in the same manner for use in determining taxonomic composition and biomass of the various components of the plankton. Rotifers and nauplii were obtained by filtering the contents of this carboy through a 40 µm Nitex screen after samples were collected and preserved for enumeration of the smaller plankton components. All samples were collected in duplicate. Macrozooplankton (cladocerans, adult copepods and copepodids) was collected with three vertical tows of a Wisconsin plankton net (20 cm diameter, 153 µm mesh).

The various plankton components were enumerated in the laboratory using standard light microscope and epifluorescent methods (Lund *et al.*, 1958; Caron, 1983; Bergstrom *et al.*, 1986). Species and community carbon biomass were determined from published relationships with body size, dry weight or biovolume. For bacteria and pico-plankton, biovolumes were converted to carbon using the factor 0.154 pg C µm⁻³ (Scavia and Laird, 1987). Ciliate biovolumes were converted to carbon using the factor 0.14 pg C µm⁻³ (Putt and Stoecker, 1989). Phytoplankton biovolumes were converted to carbon using the equation $\log C = -0.422 + 0.758 \log \text{biovolume}$ for diatoms, and $\log C = -0.460 + 0.866 \log \text{biovolume}$ for other taxa, including nanoflagellates (Strathmann, 1967). Dry weight (dw) biomass of rotifers, nauplii and macro-zooplankton was converted to C using the factor 0.48 µg C µg dw⁻¹ (Anderssen and Hessen, 1991).

Radio-tracer experiments were carried out in the laboratory under controlled conditions intended to mimic those encountered in the lake. The contents of the first carboy from each site were gently mixed and 3 l aliquots were dispensed into four glass incubation jars. Two jars were spiked with 10 µCi of ¹⁴C-HCO₃ (DIC) and two were spiked with 20 µCi of ¹⁴C-glucose (DOC). The contents were mixed and the jars were placed into a controlled environment chamber at the ambient lake water temperature and an irradiance of 150 µmol photons m⁻² s⁻¹ (representative of irradiance at the mid-depth of the euphotic zone). The jars were incubated under these conditions for 4 h, with mixing by inversion at 0.5 h intervals. The duration of incubation was based upon preliminary trials and balanced the need to incubate long enough to permit significant counts of zooplankton activity while minimizing changes in the structure and function of the community relative to that *in situ*. Immediately after incubation, the jars were placed into covered chests filled with ice and a size fractionation procedure started.

The entire contents of each jar were first filtered through successive Nitex screens of 200, 40 and 20 µm mesh size. The collected material was rinsed onto 0.45 µm Millipore filters. The filters were evacuated once, the particles were

re-suspended in approximately 50 ml of water and were then evacuated a final time (this procedure was done to minimize retention of unincorporated DIC or DOC by the filters). Filters were placed directly into plastic vials with liquid scintillation cocktail. Water passing through the first set of screens was held in the dark ice chest until further processing. This step in size-fractionating the samples was completed within 20 min. Remaining water was filtered in succession through Nuclepore 2.0 and 0.2 μm filters (using the procedure described above) and placed into additional vials of cocktail. The entire procedure was completed in 40–50 min. Scintillation vials were held in the dark for at least 30 min and then counted with a Rack Beta liquid scintillation counter. Activities were determined based on counts and filtered volumes as dpm ml^{-1} , with correction for background activity and quenching. In the experiments, relatively consistent percentages of total activity added were incorporated into particles, i.e. from 5 to 10% of the 7300 dpm ml^{-1} of added ^{14}C bicarbonate, and from 30 to 55% of the 14 700 dpm ml^{-1} of added ^{14}C glucose.

We did not carry out controls for dark uptake of ^{14}C bicarbonate or abiotic uptake of ^{14}C glucose. In previous studies of phytoplankton productivity in this lake (Havens and East, 1997), uptake in dark bottles was always less than 10% of light bottle uptake. Likewise, we find very little uptake of carbon in formalin-killed controls during routine studies of bacterial productivity.

Microscopic examination of the plankton size classes (conducted at various times during the two years prior to this study) indicates that in Lake Okeechobee, the $>200 \mu\text{m}$ size class includes $>90\%$ of the biomass of cladocerans, adult copepods and copepodids. The 40–200 μm size class includes most of the biomass of rotifers and nauplii, as well as some colonial micro-phytoplankton. The 20–40 μm size class is dominated by micro-phytoplankton, large ciliates and small rotifers. The 2–20 μm size class includes some very small rotifers (e.g. *Anuraeopsis fissa*), but is primarily composed of ciliates, phytoplankton and nano-flagellates. The 0.2–2 μm size class includes most of the biomass of pico-phytoplankton and bacterioplankton. The correspondence between operational size classes and taxonomic classes is more robust for the largest and smallest size fractions and more variable for intermediate fractions.

Carbon transfer efficiency to macro-zooplankton ($>200 \mu\text{m}$) was calculated as percent label transfer (PLT), as defined by Koshikawa *et al.* (Koshikawa *et al.*, 1996):

$$\text{PLT (\%)} = [\text{ACT}_{200} / \text{ACT}_{\text{total}}] \times 100$$

where ACT_{200} is the activity of the $>200 \mu\text{m}$ size fraction and $\text{ACT}_{\text{total}}$ is the sum of activities of all plankton size fractions. The PLT was calculated separately for the communities that were spiked with DOC versus DIC. The PLT represents the proportion of particulate carbon produced by bacteria or algae that is transferred to, and retained by macro-zooplankton during the incubation period.

Many overlapping pathways are involved in DOC and DIC transfer through the plankton web. Hence, PLT values differentiate between bacteria and algal-based carbon transfer, not between carbon transfer in the microbial loop and

Table I. Physical and chemical conditions at the near-shore and offshore sites in Lake Okeechobee on five dates when carbon tracer studies were performed. Temperatures are near-surface values and Chl *a* = integrated chlorophyll *a*

Location	Date	Depth (m)	Secchi (m)	Temperature (°C)	Chl <i>a</i> ($\mu\text{g l}^{-1}$)
Near-shore	July 98	2.8	0.5	30	36
	September 98	3.5	0.5	29	42
	February 99	2.5	0.7	16	37
	May 99	2.0	0.4	27	77
	July 99	2.5	0.4	31	50
Offshore	July 98	3.9	0.3	30	7
	September 98	4.2	0.4	28	13
	February 99	4.5	0.2	17	13
	May 99	3.5	0.3	27	20
	July 99	4.0	0.5	31	60

Table II. Carbon biomass of selected plankton components at the near-shore and offshore sites in Lake Okeechobee on the five dates when carbon tracer studies were performed. COPE = copepods, CLAD = cladocerans, MICZ = microzooplankton (rotifers, nauplii and ciliates), PHYT = micro-phytoplankton, NFLA = nanoflagellates, BACT = bacteria and picoplankton. All data have units of $\mu\text{g C l}^{-1}$

Location	Date	COPE	CLAD	MICZ	PHYT	NFLA	BACT
Near-shore	July 98	12	11	60	6100	60	790
	September 98	67	1	120	5800	80	60
	February 99	44	1	130	2600	90	100
	May 99	12	0	240	3000	160	90
	July 99	7	7	210	3600	120	160
Offshore	July 98	24	2	20	300	100	720
	September 98	140	3	80	1500	80	50
	February 99	146	2	30	300	60	70
	May 99	29	1	60	400	110	50
	July 99	9	1	80	4500	80	130

grazing food chain *per se*. Koshikawa *et al.* (Koshikawa *et al.*, 1996) noted that PLT is a time-dependent value; however, differences in the magnitude of PLT do correspond to differences in magnitude of transfer efficiencies at any given time.

The two study sites experienced a nearly 1 m depth variation, reflecting the transition from a rainy season in late summer to a dry season in winter–spring (Table I). The offshore site displayed lower Secchi transparencies on three of the five sampling dates. Water temperatures were near 30°C, except in February when temperatures were reduced to 15°C. Chlorophyll *a* concentrations were generally highest at the near-shore site.

The temporal pattern of macro-zooplankton biomass (Table II) was similar at the two sites; maxima occurred in September and February, and minima occurred in May and July. Biomass maxima were two- to threefold higher at the offshore site, despite the lower concentrations of chlorophyll *a*. Beaver and Havens concluded that this spatial difference may be caused by greater fish predation in

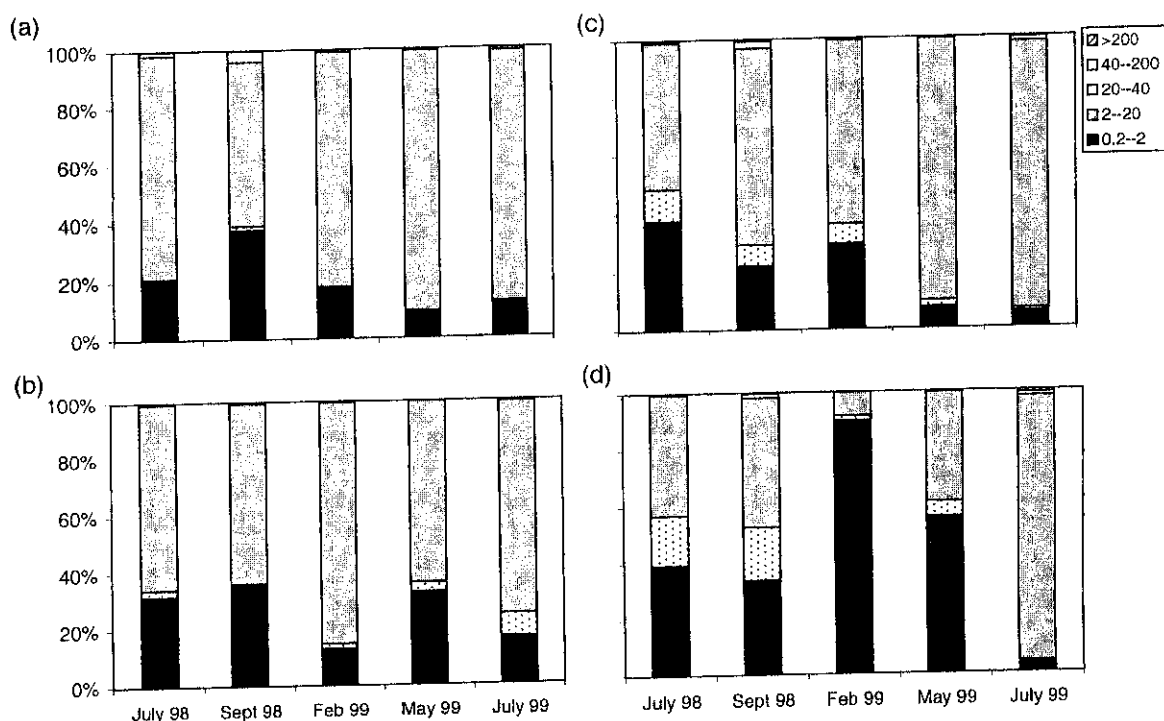


Fig. 1. Percentage of particulate activity found in five different size classes of plankton after 4 h incubations with ^{14}C -labelled (a) DOC at the near-shore site; (b) DOC at the offshore site; (c) DIC at the near-shore site; and (d) DIC at the offshore site in Lake Okeechobee.

the near-shore region (Beaver and Havens, 1997). Calanoid copepods (*Diaptomus dorsalis*) accounted for most of the macro-zooplankton biomass, except on three of 10 occasions.

At the near-shore site, most of the seston carbon was associated with phytoplankton (Table II), in particular, large filamentous and colonial cyanobacteria (CYAN). In July 1998, bacterial biomass was also high. At the offshore site, phytoplankton biomass was lower than at near-shore, and it peaked in July 1999. Microzooplankton and nanoflagellates displayed less seasonal variation in biomass and did not reach the high levels displayed by phytoplankton and bacteria.

The greatest percentage of radio-label was generally found in the 20–40 μm size class of the plankton (Figure 1A–D). In the treatment where radio-labelled carbon was added as DIC, most of this activity may reflect direct photosynthetic uptake by the micro-phytoplankton, which strongly dominated the biomass (Table II). Where carbon was added as radio-labelled DOC, high activity in the 20–40 μm size class may reflect incorporation of labelled bacterial cells into microbial grazers, including ciliates and rotifers. On two occasions (February and May 1999), a substantially greater percentage of the radio-label added as DIC occurred in the smallest (0.2–2 μm) size class.

The percent label transfer (PLT) during 4 h to the >200 μm size class (Figure 2A–B) was always low, with values ranging from <0.1 to 1.0% of the total particulate carbon uptake. A very low PLT occurred at the offshore site in February 1999, when most of the added DIC remained in the pico-plankton size class and

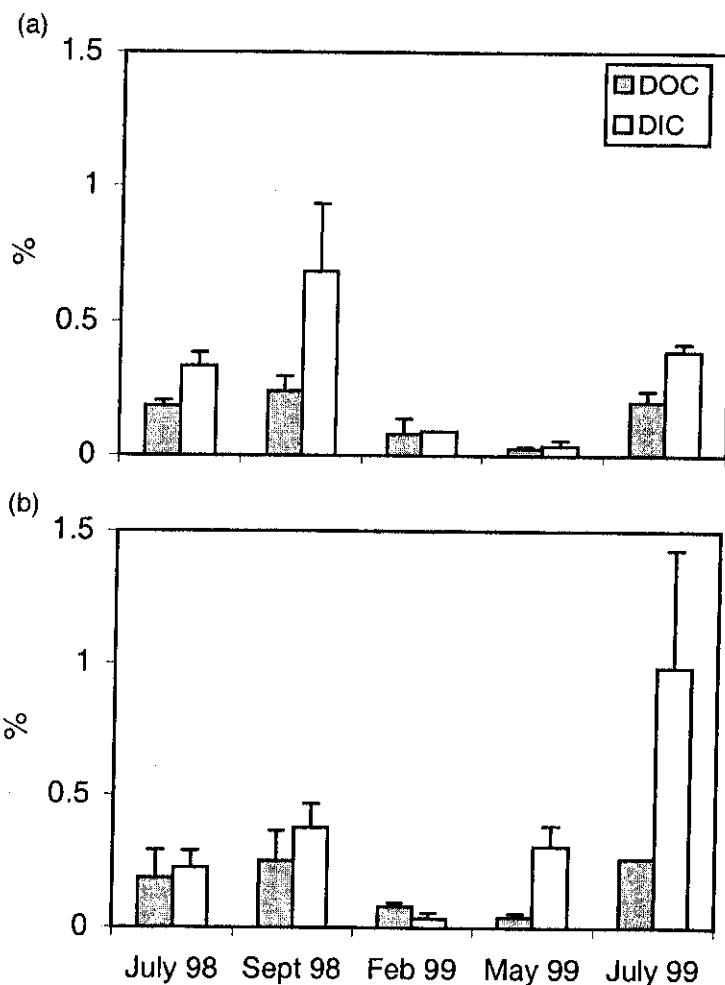


Fig. 2. Percent label transfer to the >200 µm plankton size class from DOC and DIC based pathways at the near-shore (a) and offshore (b) sampling sites in Lake Okeechobee. Vertical bars are + one standard deviation.

copepods dominated the macro-zooplankton. However, similarly low PLT occurred on other occasions when these conditions did not occur.

The PLT observed in Lake Okeechobee were similar to those found by Koshikawa *et al.* (Koshikawa *et al.*, 1996), who measured PLT after 4 h and reported values of 0.3–1.0% when copepods dominated the macro-zooplankton of the Seto Inland Sea of Japan. They measured a significantly higher PLT when the macro-zooplankton was dominated by *Doliolletta gegenbauri*, a taxon that can exploit small particles by capturing them in a net of mucus. Wylie and Currie observed that 'only a small percentage of label (carbon) is transferred to the crustacean trophic level from bacteria or algae', but they also concluded that carbon transfer from bacteria and pico-plankton is significantly greater when cladocerans dominate the macro-zooplankton compared with times of copepod dominance (Wylie and Currie, 1991). Havens (Havens, 1994) reported high values for PLT in a mesotrophic temperate lake dominated by *Eubosmina* and *Daphnia*, species that are effective in grazing pico-plankton and bacteria (Riemann, 1985). A more quantitative comparison with the present results is not possible, as

Havens only measured PLT after 24, 48 and 96 h. However, in treatments that became dominated by copepods (due to loss of cladocerans because of acid toxicity), the PLT remained very low and within the range observed here for the entire 96 h of study. This suggests that a low PLT is a real attribute of the copepod-dominated community of Lake Okeechobee, and not simply a function of short incubation time.

On all occasions except February 1999, the PLT for ^{14}C -glucose (DOC) was significantly lower (non-parametric ANOVA, $p = 0.05$) than that measured for ^{14}C -bicarbonate (DIC). These results might reflect the feeding habits of large copepods. We did observe bacterial grazing by *D. dorsalis* (Work *et al.*, submitted) but generally, these animals are more effective in exploiting larger food particles, including phytoplankton, ciliates and rotifers (Williamson and Bulter, 1986; Sanders and Wickham, 1993). A large portion of the carbon that enters a pelagic food web as bacteria may reach copepods by means of these microbial grazers. The large percentages of label observed in the 20–40 μm size class support this view. However, such pathways involve multiple trophic links and provide a greater opportunity for respiratory and other carbon losses than in two-step pathways that occur between algae and zooplankton (Sanders and Wickham, 1993).

Despite the statistical results, it is important to note that all values of PLT were between 0.1 and 1.0% (very low) and perhaps not that different from an ecological standpoint. The low efficiency in the algal-based pathway may be due in part to the seston dominance by large filamentous and colonial cyanobacteria. These large algae are found in the gut contents of calanoid copepods in Lake Okeechobee (Work *et al.*, submitted) but they may not be effectively grazed or digested (Paerl, 1988). This may further explain why a large percentage of the added label remained in the 20–40 μm size class and did not move upwards into the macrozooplankton class during the 4 h incubations.

A comparison of seasonal changes in PLT with the environmental (water temperature, chlorophyll *a*, transparency) and biological (macro-zooplankton and seston taxonomic and size structure) attributes did not indicate any consistent relationships. This probably reflects the small degree of seasonal variation in PLT in Lake Okeechobee, where transfer efficiencies were always very low. Any effects of seasonal changes in environmental conditions and community structure on PLT must be reconciled by controlled experiments.

Ducklow *et al.* studied the transfer of label from ^{14}C glucose-labelled bacteria in a marine plankton food web and concluded that bacteria are primarily a sink for carbon and not an important source of zooplankton food (Ducklow *et al.*, 1986). This conclusion was based on the finding that after 13 days of incubation, only 2% of the label taken up by bacteria occurred in the zooplankton size class. However, Sherr and Sherr (Sherr and Sherr 1987) noted that in a prior mesocosm experiment in the same ecosystem, transfer of carbon from phytoplankton to zooplankton was also very low. Koshikawa *et al.* directly compared the efficiency of carbon transfer in algal and bacteria-based food webs; they found that transfer efficiencies were low but nearly the same for both pathways (Koshikawa *et al.*, 1996). We obtained a similar result. The key point is that both major pathways of carbon flux may be relatively inefficient. However, this does not mean that they

are not important in supporting higher trophic levels. The PLT measured here and by Ducklow *et al.* (Ducklow *et al.*, 1986) and Koshiwawa *et al.* (Koshiwawa *et al.*, 1996) equates only in a relative sense with the absolute rates of carbon flux. In the case of Lake Okeechobee, our more detailed investigation of carbon dynamics (Work *et al.*, submitted) indicates that the bacteria-based pathway can transfer large quantities of carbon to zooplankton. However, both that pathway and the algal-based one may be very 'leaky' in terms of respiration and other loss processes.

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