

## SHORT COMMUNICATION

## Zooplankton grazing on bacteria and cyanobacteria in a eutrophic lake

KIRSTEN A. WORK<sup>1</sup>\* AND KARL E. HAVENS<sup>2</sup><sup>1</sup>STETSON UNIVERSITY BIOLOGY DEPARTMENT, DELAND, FL 32723 AND <sup>2</sup>SOUTH FLORIDA WATER MANAGEMENT DISTRICT, WEST PALM BEACH, FL 33416-4680, USA

\*CORRESPONDING AUTHOR: kwork@stetson.edu

*During in situ experiments in a cyanobacteria- and copepod-dominated eutrophic subtropical lake, all taxa of protozoa and metazoan zooplankton grazed fluorescently labelled bacteria and all metazoan zooplankton taxa grazed large filamentous and colonial cyanobacteria.*

The microbial loop, a pathway that links heterotrophic bacteria → protozoa → zooplankton, has been recognized for some time as an important route for carbon and energy flow (Pomeroy, 1974; Azam *et al.*, 1983; Sherr and Sherr, 1987). Bacterial carbon also may be transferred directly to macrozooplankton by certain micro-filtering cladocerans (Geller and Müller, 1981), particularly *Daphnia* spp. (Riemann, 1985). In communities lacking filter-feeding cladocerans, there is some question as to whether notable bacteria → zooplankton links exist. Calanoid copepods have been observed to consume bacteria (Knoechel and Holtby, 1986), but most studies assume that cyclopoid copepods do not [e.g. (Sanders *et al.*, 1989; Thouvenot *et al.*, 1999)]. Hwang and Heath documented bacterivory by rotifers and cladocerans in Lake Erie, but found that bacterivory was rare or absent among calanoid and cyclopoid copepods (Hwang and Heath, 1999). Most carbon dynamics models [e.g. (Wylie and Currie, 1991)] assume that there is no bacteria → zooplankton pathway when copepods (of any type) dominate the community.

The pathway from large cyanobacteria to zooplankton also is generally assumed to be energetically inefficient, if present at all. Previous studies have suggested that copepods do not consume large cyanobacteria (McNaught *et al.*, 1980), and zooplankton enclosure experiments performed in cyanobacteria-dominated lakes have indicated little or no response of the phytoplankton biomass to grazer removal (Havens *et al.*, 1996). By virtue of their large size and/or presence of a sheath, large cyanobacteria may interfere with

zooplankton food gathering, may be toxic, or may not be assimilated if ingested (Arnold, 1971). Nevertheless, some authors [e.g. (de Bernardi and Giussani, 1990)] have concluded that the question of whether cyanobacteria may be suitable food for zooplankton still is unanswered.

In this study, we examined whether zooplankton consume heterotrophic bacteria and large cyanobacteria. The study was performed at Lake Okeechobee, FL, USA (26°58'N, 80°50'W), a large (surface area 1730 km<sup>2</sup>), shallow (mean depth 2.7 m) subtropical lake. The lake has two distinct pelagic regions. The offshore region has a high total phosphorus concentration (TP ~110 µg l<sup>-1</sup>), low transparency (Secchi depth <0.5 m) and low phytoplankton biomass (chlorophyll *a* <20 µg l<sup>-1</sup>) as a result of light limitation caused by frequent re-suspension of mud bottom sediments (Phlips *et al.*, 1997). The near-shore region has lower TP (<50 µg l<sup>-1</sup>), greater transparency (Secchi depth often reaches bottom), and higher phytoplankton biomass (>40 µg l<sup>-1</sup>). Large filamentous and colonial cyanobacteria dominate the phytoplankton (Cichra *et al.*, 1995) and copepods dominate the macrozooplankton in both regions (Beaver and Havens, 1996).

Sampling was carried out in July and September 1998, and February, May and July 1999 at one site each in the offshore and near-shore regions of the lake. Heterotrophic bacterial densities were determined by epifluorescent microscopy after staining cells with acriflavine (Bergstrom *et al.*, 1986). Nanoflagellates were enumerated using the primulin staining technique of Caron (Caron, 1983), and microphytoplankton and

ciliates were enumerated using the inverted microscope technique (Lund *et al.*, 1958). Crustacean zooplankton and rotifers were counted with light microscopy in a Sedgewick–Rafters chamber.

Zooplankton grazing on heterotrophic bacteria was evaluated using bacterial cells that were isolated from the lake and then fluorescently labelled. The bacteria were incubated in 5-[4,6-(dichlorotriazin-2-yl) amino-fluorescein (DTAF) for 2 h, washed three times to remove unincorporated DTAF and sonicated to reduce clumping. The size frequency distribution of the cultured bacteria fell within that of the lake bacteria (cultured cell volume =  $0.086 \pm 0.054 \mu\text{m}^3$  versus lake cell volume  $0.21 \pm 0.33 \mu\text{m}^3$ ), although  $27 \pm 16\%$  of the cultured bacteria occurred in small clumps. At each site in the field, fluorescently labelled bacteria (FLB) were added at  $\sim 20\%$  of the ambient bacterial density to a 2 l Haney chamber containing lake plankton. The Haney chamber was incubated at mid-epilimnion ( $\sim 1$  m) for 5 min. After incubation, the contents of the chamber were filtered onto 40 and 200  $\mu\text{m}$  Nitex<sup>®</sup> screens to collect micro- and macrozooplankton fractions. The retained animals were preserved in 4% sucrose–formalin solution. An aliquot of non-preserved water passing the 40  $\mu\text{m}$  screen was placed into a dark ice chest to allow nanoflagellates to continue feeding for 60 min, after which they were preserved with cacodylate-buffered glutaraldehyde. All

organisms were examined with epifluorescence microscopy for presence of FLB in their guts or food vacuoles. We did not evaluate the consumption of cyanobacterial picoplankton because of the faint fluorescence of chlorophyll *a* through zooplankton body walls. Differences in percentage bacterivory among grazer taxa were evaluated with a Wilcoxon paired-sample test and Kruskal–Wallis non-parametric analysis of variance.

Grazing on phytoplankton was evaluated by examination of the gut contents of macrozooplankton. Ten individuals of each species were examined from each replicate sample where possible. Individual zooplankton were removed from the sample with forceps, placed on a microscope slide, examined for phytoplankton adhered to the body or caught in the carapace and then crushed with a cover slip. The cells liberated from the gut were identified to genus and counted. Selectivity of zooplankton for particular phytoplankton taxa was evaluated with the adjusted forage ratio (AFR) of Confer *et al.* (Confer *et al.*, 1990). AFR represents selectivity for food items with the equation  $r_i/p_i - 1$ , where  $r_i$  is the proportion of a food item in the diet and  $p_i$  is the proportion of the food item in the environment (e.g. lake).

Although most of the heterotrophic organisms were abundant at the near-shore site (Table I), there was no significant difference in density between sites for bacteria, nanoflagellates, all calanoid copepods, cyclopoid nauplii, *Daphnia* sp. and other cladocerans ( $P > 0.05$ ).

Table I: Densities (number  $\text{ml}^{-1}$ ) of planktonic organisms at the near-shore and offshore stations of Lake Okeechobee

Organism	Near-shore	Offshore
<b>Phytoplankton</b>		
Large cyanobacteria	$4.2 \times 10^4 \pm 1.3 \times 10^4$	$3.4 \times 10^4 \pm 5.3 \times 10^4$
Greens	$5.8 \times 10^3 \pm 1.7 \times 10^3$	$1.9 \times 10^3 \pm 2.2 \times 10^3$
Diatoms	$6.5 \times 10^3 \pm 2.5 \times 10^3$	$2.5 \times 10^3 \pm 2.2 \times 10^3$
<b>Bacteria</b>	$3.6 \times 10^9 \pm 2.3 \times 10^9$	$3.8 \times 10^9 \pm 3.3 \times 10^9$
<b>Protozoa</b>		
HNAN	$1.4 \times 10^7 \pm 8.3 \times 10^6$	$1.2 \times 10^7 \pm 3.7 \times 10^6$
ANAN	$2.0 \times 10^7 \pm 1.3 \times 10^6$	$2.2 \times 10^7 \pm 1.2 \times 10^7$
Ciliates	$55.4 \pm 31.4$	$13.6 \pm 11.2$
<b>Microzooplankton</b>		
Calanoid nauplii	$0.091 \pm 0.061$	$0.062 \pm 0.021$
Cyclopoid nauplii	$0.16 \pm 0.15$	$0.13 \pm 0.15$
Rotifers	$1.3 \pm 1.5$	$0.35 \pm 0.23$
<b>Macrozooplankton</b>		
<i>Diaptomus</i>	$1.4 \times 10^{-2} \pm 8.6 \times 10^{-3}$	$3.0 \times 10^{-2} \pm 2.3 \times 10^{-2}$
Cyclopoid copepods	$2.4 \times 10^{-3} \pm 2.1 \times 10^{-3}$	$4.6 \times 10^{-3} \pm 2.3 \times 10^{-3}$
<i>Daphnia</i> spp.	$7.3 \times 10^{-4} \pm 1.4 \times 10^{-3}$	$6.6 \times 10^{-4} \pm 5.5 \times 10^{-4}$
Other Cladocerans	$6.8 \times 10^{-3} \pm 7.0 \times 10^{-3}$	$3.4 \times 10^{-3} \pm 2.9 \times 10^{-3}$

HNAN, heterotrophic nanoflagellates; ANAN, autotrophic nanoflagellates.

Only ciliates and rotifers were significantly more abundant at the near-shore site ( $P=0.001$ , and  $P=0.02$ , respectively), whereas cyclopoid copepods were more abundant at the offshore site ( $P=0.005$ ). The phytoplankton was dominated numerically by cyanobacteria and the densities of all major divisions were higher at the near-shore site (Table I;  $P < 0.05$ ).

Every species of protozoa, rotifer and crustacean zooplankton examined in this study had FLB in their food vacuoles or guts, with the exception of *Daphnia ambigua*, which was represented by only two individuals in the analysis (Tables II and III). The percentage of animals that grazed FLB was  $>20\%$  for all taxa (Table II and III); hence the entire assemblage may be considered bacterivorous. The percentage of animals containing FLB was higher at the offshore site for crustacean zooplankton ( $P=0.001$ ), but there was no significant difference between sites for rotifers, heterotrophic nanoflagellates,

mixotrophic nanoflagellates or ciliates ( $P > 0.05$ ).

All species of crustacean zooplankton had both filamentous and colonial cyanobacteria in their guts. Species consumed included *Lyngbya* spp., *Planktothrix* (*Oscillatoria*) spp., *Anabaena circinalis* and *Microcystis aeruginosa*. Consumption of cyanobacteria was most common in *Diatomus dorsalis* ( $\sim 60\%$  of phytoplankton consumed were cyanobacteria) and *Daphnia* spp. ( $\sim 70\%$  of phytoplankton consumed were cyanobacteria). Consumption of colonial cyanobacteria generally was lower than consumption of filaments ( $9.3 \pm 10.5$  versus  $34.5 \pm 19.5\%$ ).

The relative abundance of phytoplankton taxa in the lake (Figure 1a) differed from that observed in zooplankton guts (Figure 1b,  $P \leq 0.05$ ), with the exception of *Daphnia lumholtzi* and *Ceriodaphnia* spp., whose diets closely reflected relative availability of food types. Most zooplankton consumed a disproportionately large number of diatoms at both sites (Figure 1c), and consumption

Table II: Mean percentage ( $\pm SD$ ) of protozoans that contained fluorescently labelled bacteria (FLB) following *in situ* incubation experiments in Lake Okeechobee, FL, USA

Organism	Number examined	Near-shore	Offshore
<b>Flagellates</b>			
Heterotrophic	923	46.1 $\pm$ 12.7	54.3 $\pm$ 19.5
Autotrophic	1535	18.0 $\pm$ 8.53	20.2 $\pm$ 18.0
<b>Ciliates</b>			
Order Choreotrichida			
<i>Strobilidium</i> spp.	370	87.8 $\pm$ 10.8	89.9 $\pm$ 8.43
<i>Tintinnidium</i> spp.	201	76.3 $\pm$ 28.3	92.1 $\pm$ 12.3
<i>Codonella</i> spp.	95	25.0 $\pm$ 35.4	89.8 $\pm$ 10.7
Order Oligotrichida			
<i>Strombidium</i> spp.	94	99.9 $\pm$ 0.4	100 $\pm$ 0
<i>Halteria</i> spp.	43	79.1 $\pm$ 25.1	83.3 $\pm$ 28.9
Order Haptorida			
<i>Mesodinium</i> spp.	60	77.4 $\pm$ 16.7	60.8 $\pm$ 24.0
<i>Didinium</i> spp.	6	87.5 $\pm$ 17.7	
<i>Spathidium</i> spp.	24	89.7 $\pm$ 9.0	100
<i>Askenasia</i> spp.	14	100 $\pm$ 0	75.0
Order Scuticociliatida			
<i>Cyclidium</i> spp.	307	86.1 $\pm$ 7.1	85.3 $\pm$ 11.5
Order Prorodontida			
<i>Holophrya</i> spp.	15	70.2 $\pm$ 28.2	100
<i>Urotricha</i> spp.	116	89.5 $\pm$ 6.9	80.6 $\pm$ 21.7
<i>Coleps</i> spp.	11	67.9 $\pm$ 25.2	
Order Peniculida			
<i>Paramecium</i> spp.	40	67.4 $\pm$ 9.2	87.5 $\pm$ 17.7
Order Protostomatida			
<i>Vasicola</i> spp.	28	47.2 $\pm$ 29.2	100
Order Sesselida			
<i>Vorticella</i> spp.	54	96.9 $\pm$ 6.25	83.3

Table III: Mean percentage ( $\pm$ SD) of zooplankton species with fluorescently labelled bacteria in their guts

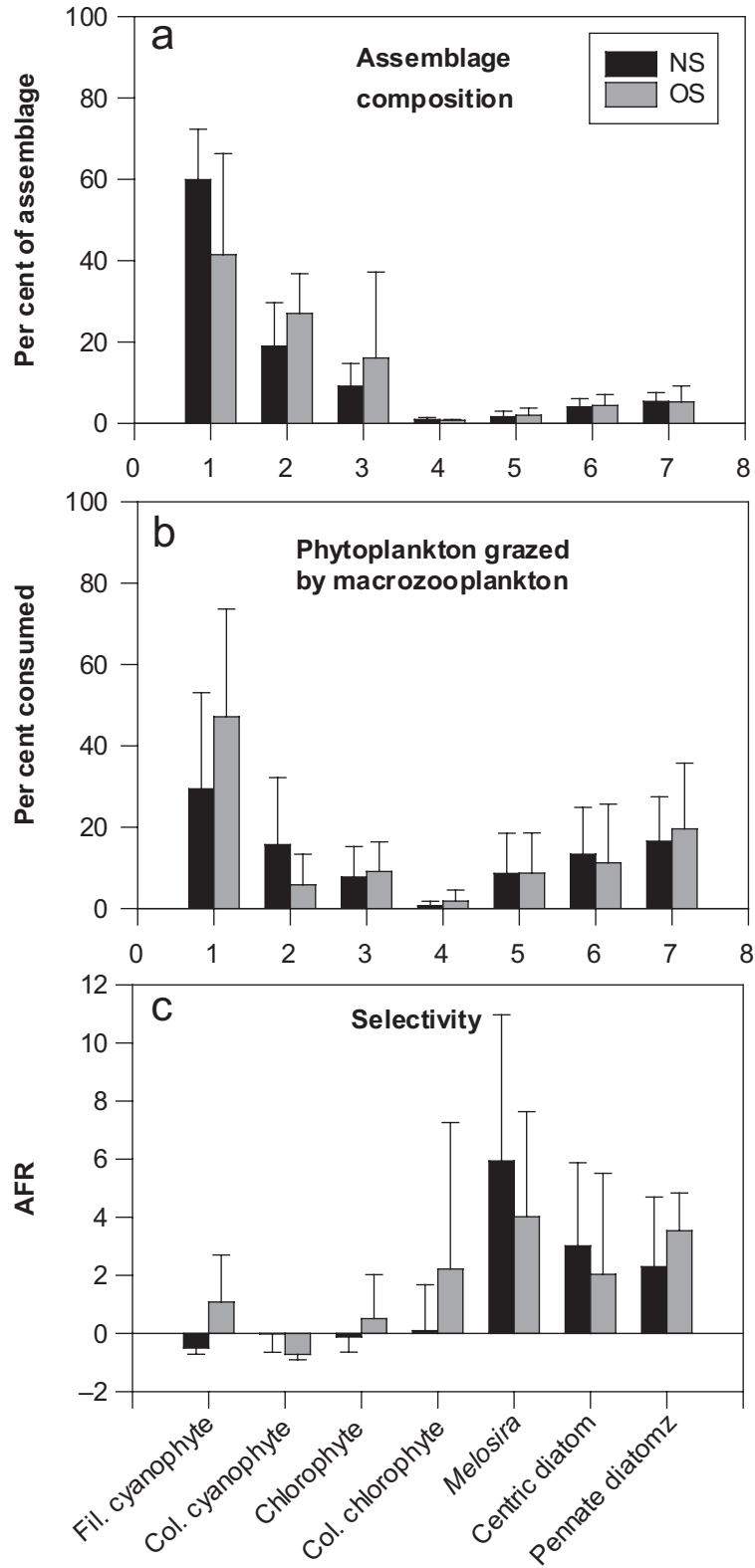
Organism	Number examined	Near-shore	Offshore
<b>Microzooplankton</b>			
Calanoid nauplii	624	35.7 $\pm$ 19.7	40.7 $\pm$ 16.5
Cyclopoid nauplii	373	21.0 $\pm$ 17.3	24.7 $\pm$ 17.5
<i>Anuraeopsis fissa</i>	3	66.7	
<i>Asplanchna herricki</i>	22	40.0 $\pm$ 56.6	16.7
<i>Brachionus angularis</i>	52	71.7 $\pm$ 43.0	100
<i>Brachionus calyciflorus</i>	11	90.0 $\pm$ 14.1	
<i>Brachionus havanaensis</i>	143	98.5 $\pm$ 3.4	83.3 $\pm$ 28.9
<i>Colurella</i> sp.	6	100	
<i>Conochiloides dossuarius</i>	22	80.0	46.5 $\pm$ 65.7
<i>Conochilus unicornis</i>	89	90.6 $\pm$ 12.0	100 $\pm$ 0
<i>Epiphanes senta</i>	7	67.9 $\pm$ 25.2	
<i>Filinia longiseta</i>	2	0	100
<i>Gastropus hyptopus</i>	4	100 $\pm$ 0	
<i>Hexarthra mira</i>	5	100	25
<i>Keratella cochlearis</i>	326	73.0 $\pm$ 19.3	79.8 $\pm$ 14.6
<i>Keratella testudo</i>	70	43.7 $\pm$ 38.8	35.0 $\pm$ 49.5
<i>Keratella quadrata</i>	5	100	
<i>Lecane bulla</i>	4	50.0	
<i>Lecane luna</i>	9	66.7 $\pm$ 57.7	
<i>Philodina</i> sp.	13		100
<i>Polyarthra major</i>	68	0	28.3 $\pm$ 18.9
<i>Polyarthra vulgaris</i>	195	25.0 $\pm$ 35.4	42.5 $\pm$ 18.6
<i>Trichocerca multicrinis</i>	5	16.7 $\pm$ 28.9	
<i>Trichocerca pusilla</i>	7	33.3 $\pm$ 47.1	25
<i>Trichocerca rousseleti</i>	5	75.0	0
<i>Trichocerca similis</i>	2	25.0 $\pm$ 35.4	
<b>Macrozooplankton</b>			
<i>Diaptomus dorsalis</i>	100	39.6 $\pm$ 27.9	92 $\pm$ 11.4
<i>Mesocyclops edax</i>	2		50.0
<i>Tropocyclops prasinus</i>	76	32.0 $\pm$ 11.2	47.0
Calanoid copepodites	116	39.8 $\pm$ 24.0	66.7 $\pm$ 57.7
Cyclopoid copepodites	134	17.0 $\pm$ 5.9	32.0 $\pm$ 8.5
<i>Bosmina longirostris</i>	17	26.7 $\pm$ 46.2	33.0
<i>Ceriodaphnia reticulata</i>	5	100	
<i>Daphnia ambigua</i>	2	0	0
<i>Daphnia lumholtzi</i>	2	100 $\pm$ 0	
<i>Diaphanosoma brachyurum</i>	4	100 $\pm$ 0	100

of cyanobacteria was low relative to availability. However, the zooplankton consumed a higher proportion of filamentous cyanobacteria at the offshore site where the density of chlorophytes and diatoms was low.

In summary, zooplankton consumption of bacteria and cyanobacteria (filamentous and colonial) was extensive in this eutrophic subtropical lake. Despite previously published taxonomic designations of feeding preference [e.g. (Burns, 1968; McNaught *et al.*, 1980; Williamson

and Butler, 1986; Sanders *et al.*, 1989; Sanders and Wickham, 1993; Ooms-Wilms, 1997)], our results indicated that all planktonic taxa, including a wide range of flagellates, ciliates, rotifers, cladocerans and copepods, can graze bacteria and that all macrozooplankton can graze filamentous and colonial cyanobacteria, although diatoms may be preferred.

Several mechanisms may have contributed to the frequent consumption of heterotrophic bacteria and



**Fig. 1.** Selective grazing of phytoplankton taxa by macrozooplankton. The ambient percentages of phytoplankton taxa in the Lake Okeechobee assemblage (a) differed from the percentages of phytoplankton taxa grazed by macrozooplankton (b). The adjusted forage ratio (AFR) indicated that macrozooplankton fed selectively on the available phytoplankton taxa (c).

cyanobacteria in Lake Okeechobee. First, zooplankton may be unable to avoid consuming bacteria and cyanobacteria in lakes in which populations of these organisms are particularly dense, i.e. consumption may be incidental, rather than intentional. Secondly, a paucity of food resources usually considered as 'edible' (e.g. small chlorophytes and cryptophytes) may require zooplankton to supplement their diet with less favourable foods such as bacteria and cyanobacteria. Thirdly, populations of zooplankton that co-occur with dense cyanobacterial populations may be better able to digest cyanobacteria than unexposed zooplankton (Hairston *et al.*, 1999). Regardless of the mechanism, consumption of bacteria and cyanobacteria may be a common phenomenon in other lakes with dense cyanobacteria and a low abundance of other food resources. However, it remains to be seen whether these same food items can serve as a major carbon and energy source, since our study considered only ingestion and not assimilation of the bacterial and cyanobacterial taxa.

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