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Difference in the Bacterial Utilization Ability of Four Cladoceran Plankton (Crustacea: Cladocera)

Jotaro Urabe[1] and Yasunori Watanabe[2]

1) Natural History Museum and Institute, Chiba
955-2 Aoba-cho, Chiba 280, Japan
2) Department of Biology, Tokyo Metropolitan University
Fukazawa 2-1-1, Setagaya-ku, Tokyo 158, Japan

Abstract Ingestion and assimilation rates on a strain of bacteria isolated from a reservoir and on a green alga, *Chlamydomonas reinhardi*, were examined for four common cladocerans, *Daphnia galeata, Diaphanosoma brachyurum, Bosmina longirostris* and *Bosminopsis deitersi*, by labeling the foods with ^{14}C. The cladocerans showed differential ability to utilize the bacteria as a food source. *D. galeata* and *D. brachyurum* ingested the bacteria as well as the algae but bacterial ingestion rates of *B. longirostris* and *B. deitersi* were much lower than the algal ingestion rate. Among the cladocerans, only *D. brachyurum* assimilated the bacteria as efficiently as the algae, assimilation efficiency for the bacteria was less than one third of that for the algae in other species. These results suggest that the relative importance of direct nutritional flow from bacteria to zoo plankton differs among food webs with different zooplankton compositions.

Key words : cladocera, zooplankton, bacteria, ingestion rate, assimilation rate, nutritional flow, pelagic food web.

Classically, a nutritional flow from algae to zooplankton, which in turn are preyed upon by higher consumers such as fish, has been thought to be the dominant trophic channel in the pelagic food web in lakes and ponds. However, recent studies suggest the importance of an alternative trophic channel, the nutritional flow from free-living bacteria to higher consumers through bacterial feeding of zooplankton (Gophen *et al.*, 1974; Porter, 1984; Nagata, 1986). This view comes from several lines of evidence. First, algal production in some lakes is insufficient to support zooplankton production (Nauwerk, 1963; Kankaala, 1988). Second, the biomass of free-living bacteria sometimes contributes a considerable proportion of the suspended organic matter (Sorkin and Paveljeva, 1978; Nagata, 1986). Finally, some freshwater zooplankton species can ingest bacteria as efficiently as they can algae (Gophen and Geller, 1984; Brendelberger, 1985; DeMott, 1985; Hessen, 1985; Nagata and Okamoto, 1988).

However, in order to evaluate the relative importance of these channels in a pelagic food web, it is necessary to examine the ability of the zooplankton to assimilate bacteria as well as ingest them. If zooplankton can ingest bacteria in substantial amounts, but assimilate them poorly, the nutritional flow from bacteria to zooplankton would be less important than that from algae. Furthermore, if the abilities of bacterial ingestion and assimilation differ among zooplankton species, the relative importance between the trophic channels would change with changing zooplankton composition. This implies that zooplankton composition may be influenced by the relative abundance of algal and bacterial biomasses, since the zooplankton frequently meet food shortages in their natural environment (e.g. Lampert, 1985). Information on the ability of freshwater zoo plankton to assimilate bacteria is however very limited (Gophen *et al.*, 1974; Pace *et al.*, 1983).

The purpose of this study was to examine whether zooplankton can assimilate bacteria as well as algae, and whether the ingestion and assimilation abilities for bacteria are the same among four cladoceran species, *Daphnia galeata* G. O. Sars, *Diaphanosoma brachyurum* (Lievin), *Bosmina longirostris* (Müller) and *Bosminopsis deitersi* Richard, the dominant zooplankton in many lakes and ponds. We evaluated these abilities by comparing the ingestion and assimilation rates for a bacterial strain isolated from a reservoir to those for a green alga, *Chlamydomonas reinhardi*. The ingestion and assimilation rates were estimated using a radioisotope technique

(Lampert, 1977; Lynch *et al.*, 1986).

Materials and Methods

The bacterium used was isolated from Ogochi Reservoir (Lake Okutama), located in the northwestern part of Tokyo, one month before the experiments and cultivated in 1/4 PYG medium (Konda and Tezuka, 1979). It was not identified, but was short rod and ranged from 1.5 to 3.0 μm in diameter after cultivation. A green alga, *Chlamydomonas reinhardi* Dangeard (IAM strain C-238), was grown axenically on modified Bristol medium (Watanabe, 1960) and used for the experiments. The carbon content of the bacterium and *C. reinhardi* was determined using a CHN analyzer (Yanagimot MT-3), yielding means of 0.84 and 25 pg C cell^{-1}, respectively.

The four cladocerans, *Daphnia galeata, Diaphanosoma brachyurum, Bosmina longirostris* and *Bosminopsis deitersi,* were collected from Ogochi Reservoir four days before the experiments and kept in 1-L beakers containing filtered (0.45-μm) reservoir water with the cultured bacteria (3×10^5 cells ml^{-1}) and *C. reinhardi* (1×10^4 cells ml^{-1}) as foods at room temperature (20°C). In the stock cultures, food and water were changed every day.

In all experiments, the food concentration was set at 0.5 μg C ml^{-1}, corresponding to 6×10^5 cells ml^{-1} in the bacteria and 2×10^4 cells ml^{-1} in *C. reinhardi,* and the temperature was kept at 20°C. The cell concentration was determined by hemacytometer. Two days before the experiments, *C. reinhardi* cells from a 5-day-old culture were centrifuged, resuspended in 200 ml of fresh algal medium, and labeled with 80-100 μCi NaH $[^{14}C]O_3$ on a shaking table under fluorescent light, so that the specific activity of labeled algae in the experimental feeding suspensions was about 1×10^4 d.p.m. The bacterial cells from a 3-day-old culture were also centrifuged, resuspended in 200 ml of fresh bacterial medium and labeled with 100-120 μCi [^{14}C]-acetate on a shaking table, so that the specific activity of labeled bacteria in the experimental feeding suspensions was slightly higher than that of algae. Stock suspensions of algae or bacteria for prefeeding were also incubated in radioisotope-free media. After 48h, both non-labeled and labeled cells were centrifuged, washed twice and resuspended in filtered (0.2μm) reservoir water. Microscopy revealed that neither algal nor bacterial cells were aggregated but were suspended individually.

Experimental cladocerans were pipetted from stock cultures into 1-l flasks containing 400 ml of non-radioactive feeding suspensions. In each experimental flask, 10 to 25 individuals of each cladoceran species were placed. At 0.5 h of acclimatization, 400 ml of radioactively labeled food was added gently to the flasks to avoid any damage to the animals (DeMott, 1982). In experiments for measurement of ingestion rates, the cladocerans were allowed to feed for 9 min in radioactive feeding suspension. During the incubation, two 2-ml aliquots of the feeding suspension were sampled and filtered through 0.2-μm Sartorius filters to determine the radioactivity of the food. The entire contents of the flask were concentrated into a 30-ml bottle with a 3×3 net-covered window (96-μm mesh) on the side wall. Immediately afterwards, CO_2-enriched water was poured into the bottle to stop the feeding. The anesthetized cladocerans were rinsed with 0.001 N HCl and thereafter with distilled water, and fixed in a formalin-sucrose solution (Haney and Hall, 1973).

Assimilation rates were measured according to Lampert (1977): experimental cladocerans were allowed to feed on the labeled foods for 1 and 3 h, and the assimilation rate was estimated from the difference in radioactivity of cladocerans between 1 and 3 h. This experimental protocol eliminates the need for clearing the gut after feeding labeled food and thus avoids losses of tracer from the metabolic pool (Lampert, 1975). During the feeding of the cladocerans, experimental flasks were stirred gently once every 10 min to keep the food particles homogeneous in the suspension. At the end of the feeding period, the cladocerans were harvested as above.

Between 10 to 24 h after the experiments, the cladocerans were sorted by size and species under a dissecting microscope. According to their size and species, 1 to 4 individuals of cladocerans were placed in a scintillation vial containing 0.2 ml Protosol (New England Nuclear). After digestion of the tissues at 50°C for 24 h, each vial was filled with 10 ml of toluene-base scintillation cocktail and 0.2 ml of glacial acetic acid was added to neutralize the solubilizer-Protosol (DeMott,

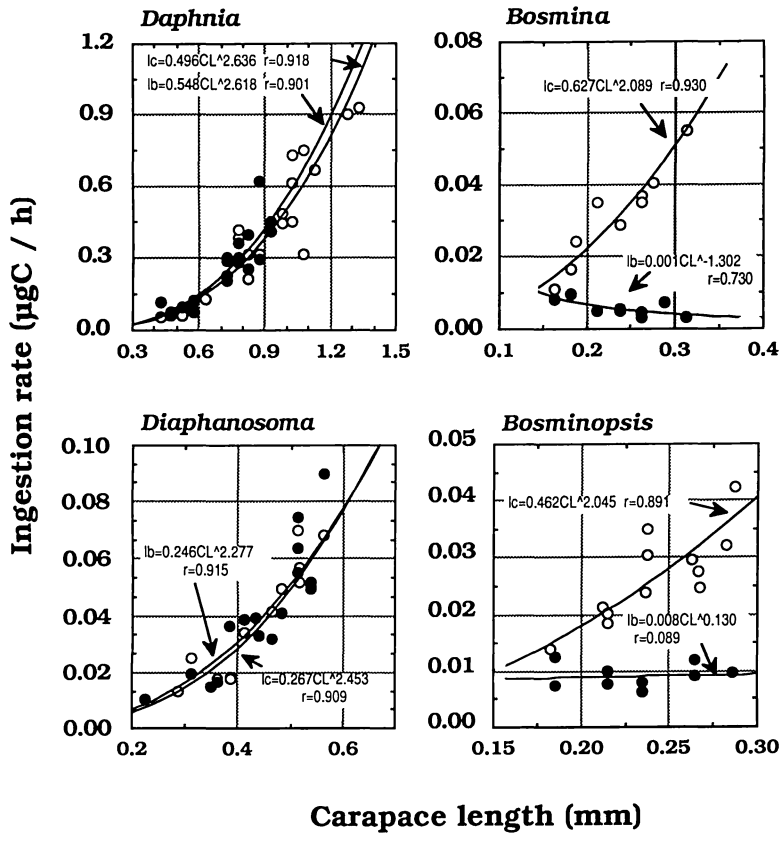

Fig. 1. Algal (Ic: clear circle) and bacterial (Ib: solid circle) ingestion rates of four cladocerans plotted against carapace length.

1982). The 0.2-μm filter with 2 ml of feeding suspension was transferred to the scintillation vial immediately after each experiment and treated in the same way as the sample of cladocerans. Radioactivity was determined by a liquid scintillation counter. The correction for quenching was made by the channels-ratio method.

Ingestion (I: μg C h^{-1}) and assimilation (A: μg C h^{-1}) rates were calculated as follows:

$$I = f \times dpm_C / (dpm_f \times t \times \alpha)$$

$$A = (A_3 - A_1) / 2$$

$$A_3 = f \times dpm_3 / [dpm_f \times \alpha \times (1-\beta_3)]$$

$$A_1 = f \times dpm_1 \times / [dpm_f \times \alpha \times (1-\beta_1)]$$

where f is food concentration, 0.5 μg C ml^{-1}; t is feeding time, 0.15; dpm_C, dpm_3 and dpm_1 are dpm per cladoceran after the feeding period of 0.15, 1 and 3 h, respectively; dpm_f is dpm per food particles in 1 ml of the food suspension; α is the correction factor for the loss of label during storage; A_3 and A_1 are incorporation rates of cladocerans during the feeding period of 3 and 1 h; β_3 and β_1 are the correction factors for loss of label due to respiration of cladocerans during the feeding period of 3 and 1 h.

It is known that 40-60% of ^{14}C incorporated by cladocerans is lost during storage longer than 10 h (Lampert and Taylor, 1985; DeMott, 1985; Nagata and Okamoto, 1988). In this study, therefore, a value of 0.5 was used as the correction factor, α. Another correction factor β is also important in estimation of the assimilation rate because cladocerans use some of the assimilated ^{14}C immediately for metabolic activity and release as $^{14}CO_2$ (Lampert, 1975). This results in a considerable underestimation of the incorporation rate for ^{14}C if feeding in labeled suspension is long. Lynch *et al.* (1986) reported that losses of ^{14}C by respiration of cladocerans were 15.3 and 23.9-25.8% of the total incorporation at 1 and 3 h, respectively. Very similar values were also

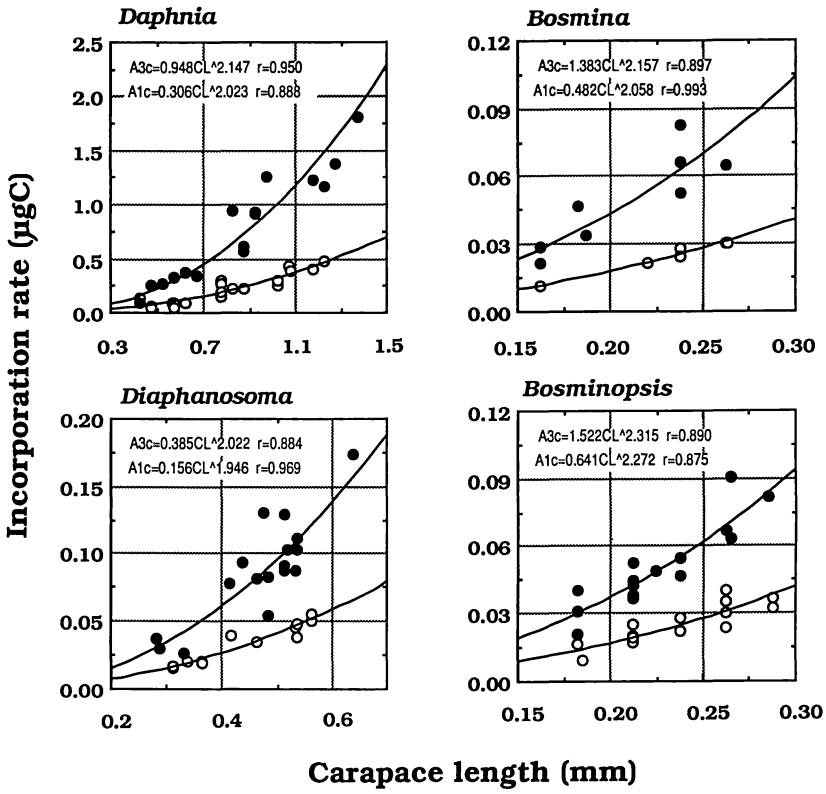

Fig. 2. Incorporation rates for labeled algae at 3h (A3c: solid circle) and 1 h (A1c: clear circle).

obtained by Lampert (1977). Values of 0.15 and 0.25 were therefore used as the respective correction factors, β_1 and β_3. The correction factors used here may be rough estimates. However, since we labeled both algae and bacteria by the same radioisotope (^{14}C), it is possible to compare ingestion and assimilation rates for bacteria and algae.

Results

In all cladocerans, the ingestion rate for *C. reinhardi* was correlated significantly with carapace length and increased with the power 2.0-2.6 of the carapace length (Fig. 1). Ingestion rates of *D. galeata* and *D. brachyurum* for bacteria were also correlated significantly with the carapace length and closely similar to those for *C. reinh ardi* However, the bacterial ingestion rate was negatively correlated in *B. longirostris* and a significant change with the carapace length was not found for bacterial ingestion rate of *B. deitersi.* In these two cladocerans, bacterial ingestion rates remained low regardless of the carapace length. The correlation analyses demonstrated that the feeding ability for bacteria differed among the cladocerans.

A significant correlation was also found between incorporated algal carbon and carapace length (Fig. 2). In all cladocerans, the slope of the regression against carapace length was nearly identical between 1 and 3 h, and the elevation at 3 h fell within a range from two to three times higher than that at 1 h. A similar trend was also detected for incorporated bacterial carbon at 1 and 3 h in *D. galeata* and *D. brachyurum* (Fig. 3). However, incorporated bacterial carbon did not show a significant increase with increasing carapace length in *B. longirostris* and *B. deitersi,* although the incorporated carbon at 3 h was slightly higher than that at 1 h.

The assimilation rate estimated from the difference in incorporated carbon between 1 and 3 h is shown in Fig.4. In *D. brachyurum,* the assimilation rate for bacteria increased with carapace length, and was close to that for *C. reinhardi* regardless

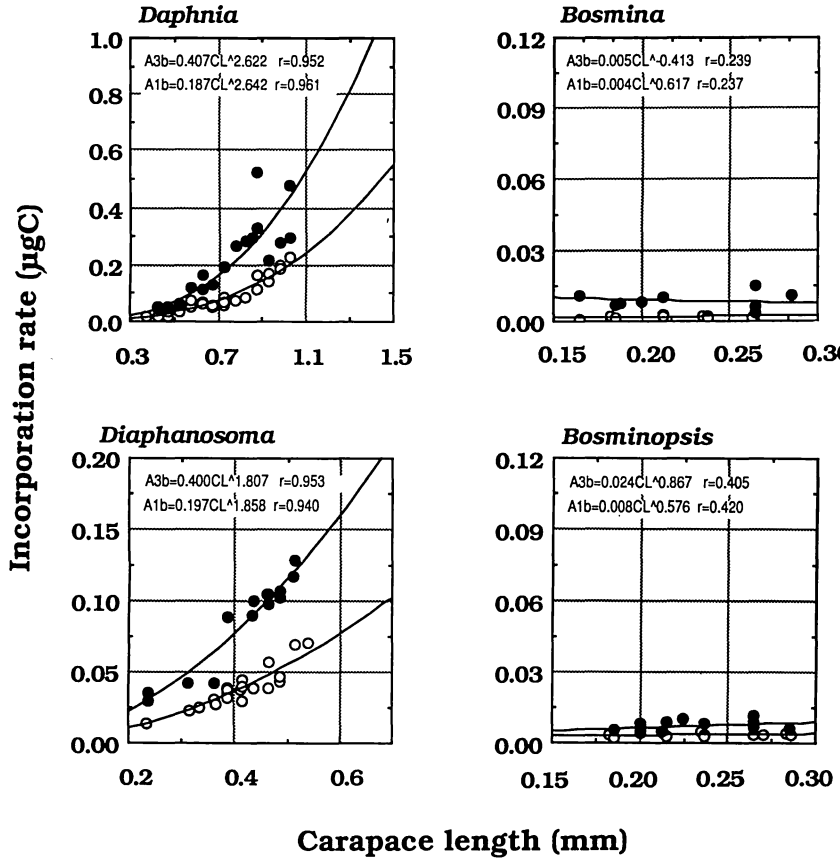

Fig. 3. Incorporation rates for labeled bacteria at 3h (A3b: solid circle) and 1 h (A1b: clear circle).

of the carapace length. The assimilation rate of *D. galeata* for bacteria also increased with carapace length, but was lower than that for the algae. *B. longirostris* and *B. deitersi* did not show any functional change in the bacterial assimilation rate with carapace length. In these two species, the bacterial assimilation rate was very low compared to the algal assimilation rate.

Assimilation efficiency (assimilation rate/ingestion rate) was somewhat different depending on the carapace length within a cladoceran. However all cladocerans assimilated more than 50% of the algae ingested (Table 1). On the other hand, the assimilation efficiency for the bacteria differed among the four species. *D. brachyurum* assimilated the bacteria as well as the algae. The bacterial assimilation efficiency of *B. longirostris* and *B. deitersi* was very low, although the former showed the highest assimilation efficiency for *C. reinhardi*. In *D. galeata,* the assimilation efficiency for bacteria was less than a third of that for the algae, although this species ingested the bacteria as efficiently as it did the algae.

Discussion

The present study demonstrated that cladocerans have different abilities to utilize free-living bacteria as a food resource, and do not necessarily assimilate the bacteria as well as algae. Of the four cladocerans examined here, two, *Bosmina longirostris* and *Bosminopsis deitersi,* did not show an increase of bacterial ingestion rate as a function of carapace length even though the algal ingestion rate increased. This implies that the ability of these species to feed on the bacteria decreases with increasing body size. The same result was also obtained for *B. longirostris* by DeMott (1982, 1985). Since the bacterial ingestion rate was much lower than the algal ingestion rate, *B. longirostris* and *B. deitersi*

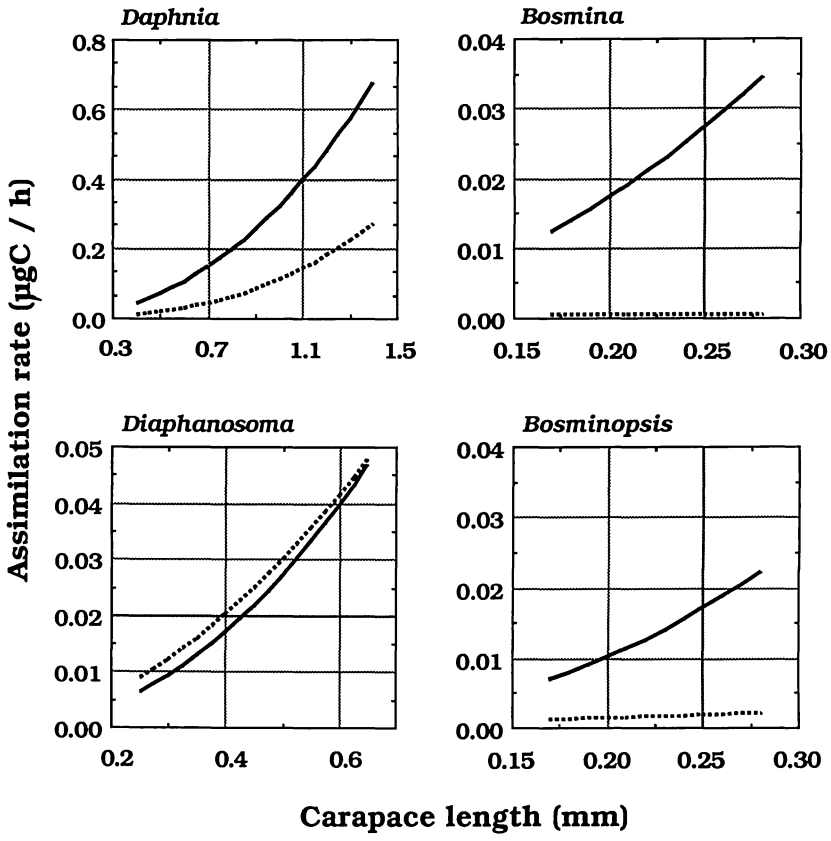

Fig. 4. Algal (solid line) and bacterial (dashed line) assimilation rates of four cladocerans.

can be considered as inefficient bacterial feeders.

The other cladocerans, *Daphnia galeata* and *Diaphanosoma brachyurum,* ingested the bacteria at the same rate as for *C. reinhardi.* The result for *D. brachyurum* coincides with a previous study (Hessen, 1985). However, Urabe (in press) showed that the feeding rate of *D. galeata* for natural bacteria is less than that for algae. The high bacterial ingestion rate of this cladoceran in the present study is probably due to the use of cultured bacteria. It is known that the feeding rate of cladocerans differs with food size and usually decreases with decreasing food size below 1μm (Hessen, 1985; Gophen and Geller, 1985). Since the cell size of the cultured bacteria used here was larger than 1.5μm, *D. galeata* could ingest the bacteria efficiently.

The value of bacteria as a food source apparently differs among cladocerans. *D. brachyurum* could assimilate the bacteria as efficiently as the algae while the assimilation efficiency of the other cladocerans for the bacteria was less than a third of that for the algae. These results suggest that, even if *D. galeata* can ingest bacteria efficiently, the contribution of bacteria as nutritional resource for *D. galeata* is small compared to that of algae. Pace *et al.* (1983) also showed that some daphnid populations cannot grow even with a

Table 1. Assimilation efficiencies of four cladocerans for *Chlamydomonas* and bacteria.

Species	*Daphnia galeata*	*Bosmina longirostris*	*Diaphanosoma brachyurum*	*Bosminopsis deitersi*
Carapace length	0.40～1.40mm	0.17～0.28mm	0.30～0.65mm	0.17～0.28mm
Chlamydomonas	56.0～85.5%	79.5～80.8%	50.5～67.4%	55.7～64.8%
Bacteria	19.3～20.4%	5.3～10.8%	51.6～76.9%	11.3～21.8%

sufficient density of natural bacteria. Urabe (in press) has provided evidence that bacterial feeding by *D. galeata* may be an incidental event associated with algal feeding.

Based on the morphology of feeding appendages and bacterial feeding rate, some authors have divided planktonic cladocerans into three groups, high efficient bacterial feeder, low efficient bacterial feeder and macrofiltrator (Geller and Müller, 1981; DeMott, 1985). This classification is valuable with respect to the grazing impact of zooplankton on bacteria, but gives less insight to the relative importance of direct nutritional flow from bacteria to high consumers via zooplankton. The present study provides alternative criteria to divide cladocerans into ecological groups, which address the function of bacteria in a given food web. The classification is made based not only on the ability of bacterial feeding, but also on assimilation efficiency for bacteria as follows:

(1) inefficient bacterial grazers, which do not ingest and assimilate bacteria efficiently (*Bosmina longirostris, Bosminopsis deitersi*);

(2) efficient bacterial grazers, which ingest bacteria but do not assimilate them efficiently (*Daphnia galeata*);

(3) efficient bacterial feeders, which ingest bacteria and assimilate them efficiently (*Diaphanosoma brachyurum*).

This classification gives the following view for pelagic food webs. In lakes and ponds where cladocerans of group (3) dominate, free-living bacteria and zooplankton would be related to each other closely with respect to nutritional flow and food web linkage (*sensu* Paine 1980). In lakes and ponds where cladocerans of group (2) dominate, the size of the bacterial population would be influenced by grazing pressure, but direct nutritional flow from free-living bacteria to zooplankton would be small compared with nutritional flow from algae even in an environment with a high bacterial biomass. On the other hand, in lakes and ponds where cladocerans of group (1) dominate, the population size of the bacteria would be little influenced by grazing pressure of zooplankton and the direct contribution of the trophic channel starting from bacteria via zooplankton would be small to production of higher consumers. Thus, the relative importance between trophic channels starting from bacteria and algae and interaction strength between bacteria and zooplankton in a given lake would be evaluated by examining the relative abundance of the zooplankton groups as well as the proportion of the bacterial biomass to suspended organic matter.

In the present study we estimated the ingestion and assimilation rates for cultured bacteria under experimental conditions. The results may therefore not be directly applicable to natural communities. Nonetheless they suggest that the relative importance of direct nutritional flow from bacteria to zooplankton differs among food webs with different zooplankton compositions. Future studies will need to estimate these rates in natural environments. Such studies will provide further information on the nature of trophic interaction between zooplankton and bacteria in the pelagic environment.

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浮遊性枝角類(Crustacea : Cladocera) 4種のバクテリアに対する摂食・同化能力

占部城太郎[1)]・渡辺泰徳[2)]

1)千葉県立中央博物館
〒280 千葉市青葉町955-2
2)東京都立大学理学部生物学教室
〒158 世田谷区深沢2-1-1

多くの湖沼に動物プランクトンとして卓越して出現する枝角類4種, *Daphniagaleata, Diaphanosoma brachyurum, Bosmina longirostris, Bosminopsis deitersi* のバクテリアと藻類に対する摂食および同化速度を ^{14}C によるラジオアイソトープ法を用いて調べた. 実験には, 藻類として *Chlamydomonas reinhardi* を, またバクテリアは野外から分離し培養した株を用いた. いずれの枝角類も藻類に対する摂食速度は高く, 同化効率は50%以上であった. しかしバクテリアに対する摂食・同化能力は種類により異なっていた. すなわち*D. brachyurum* は藻類の場合と同じようにバクテリアを効率よく摂食し同化した. 一方*D. galeata* では, バクテリアに対する摂食速度は藻類の場合と変わらないものの, 同化効率は藻類の⅓以下であった. また他の2種はバクテリアをほとんど摂食しなかった. 以上の結果から, 湖沼における高次捕食者に対する生食連鎖と腐食連鎖の相対的重要性は, 動物プランクトンの種構成によって異なっていることが示唆された.