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Filter Mesh-Sizes of *Daphnia longispina* and Its Filtering Rates on Natural Bacteria*

By

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Abstract. Diameter of setules (ϕ), intersetular distance (D) and mesh-size (M) of the filtering combs of *Daphnia longispina* collected in Lake Biwa were determined with a scanning electron microscope. The relations of each parameter to the body length (L in mm) of daphnids were; $\phi(\mu m)=0.130\ L^{0.40}$ (r=0.78; n=14), $D(\mu m)=0.402\ L^{0.40}$ (r=0.88; n=14) and $M(\mu m)=0.270\ L^{0.40}$ (r=0.82; n=14). The range of the mesh-size of *D. longispina* (L, 0.4–1.3 mm) was 0.17–0.36 μm. The filtering rate of *Daphnia* (L, 1.0–1.3 mm) on natural bacteria in 3 μm-filtered lake water and its dilution, measured by direct bacterial count method, was in the range of 0.3–0.9 ml·anim^{-1}·h^{-1} (mean, 0.57). From these results, *D. longispina* in this lake was characterized as a feeder which can efficiently ingest natural bacteria throughout its whole life stages.

Introduction

Some *Daphnia* species have been known as feeders which ingest planktonic bacteria from the feeding experiments using both cultured (Tezuka 1971; Gophen et al. 1974) and natural free-living bacteria (Peterson et al. 1978). Recently, Geller and Müller (1981) measured the mesh-sizes of the filtering combs of various cladocerans using a scanning electron microscope (SEM) and estimated the lower size limit of food spectrum of each species. Based on the ability to utilize bacteria as food, they categorized the cladoceran species into following three groups; high efficiency bacteria feeders, low efficiency bacteria feeders and macrofiltrators. They hypothesized that high efficiency bacteria feeders appear in eutrophic lakes where bacterial production is high.

In the north basin of Lake Biwa (mesotrophic), it has been reported that *Daphnia longispina*, which is one of the most dominant zooplankters in this lake, shows feeding selectivity for small size phytoplankton passing through 10 μm-screen (Okamoto 1984). However, the feeding of *D. longispina* on bacteria has never been examined in this lake. In the present work, the mesh-size of the filtering combs and the filtering rate of *D. longispina* on natural free-living bacteria were measured.

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Materials and Methods

For the scanning electron microscopic observation on the filtering combs, *D. longispina* was sampled at a pelagic region of the north basin of Lake Biwa on 13 October, 1983 and 10 January, 1984. Sampled *Daphnia* was fixed and preserved with sugar formalin. After post fixation by 2% glutaraldehyde (buffered with 0.1 M Na cacodylate) overnight, the specimens were dehydrated by ethanol series, and dried by a critical point dryer. They were then dissected and coated with Au by an ion-coater, and observed under an SEM (JEOL-JSM-25). Diameter of setule (ϕ; measured just above the basal swelling part of a setule) and intersetulal distance (D; a distance between the central axes of neighboring setules at their basal part) in filtering combs were measured on the enlarged SEM photographs (78 images were examined in total). The filtering mesh-size (M) was defined as $M=D-\phi$ (Geller and Müller 1981). When the filtering combs of both III and IV thoracic limbs of one individual were measured, no significant difference of the measured parameters of the two limbs was found. Then the data of both limbs were pooled in the analysis. From 3 to 13 randomly selected images of the combs were examined for an individual, and the standard error of each parameter was usually below 10% of the mean. Body length of a daphnid was measured as a distance from the center of the eye to the base of the spine.

Filtering rate of *D. longispina* on natural bacteria was measured principally according to Peterson et al. (1978). *Daphnia* was collected at the lake center of the south basin 1 hour before the experiments (2 November 1982). Three to five daphnids (body length, 1.0–1.3 mm) were sorted and placed into an experimental flask with 40 ml of 3 μm (Nuclepore filter) filtered lake water containing mainly free-living bacteria after 1 hour acclimation in 0.2 μm filtered lake water (particle free water). The flasks were incubated in the dark at the field temperature (17°C) and subsamples for the bacterial count were withdrawn after 0, 1, 2 and 4 hours. Also the filtering rate was determined at low bacterial densities. The low densities were prepared by dilution of 3 μm-filtrate to 1/2, 1/4 and 1/10 fold with the 0.2 μm-filtrate. In the time course experiments, exponential decrease in bacterial number was detected with significance ($P<0.05$). No change in bacterial number was found in the *Daphnia*-free controls containing either the 3 μm-filtrate or the 1/2-dilution, but significant increase was found in both the controls of 1/4- and 1/10-dilution series. In the former case, the filtering rate was calculated according to Peterson et al. (1978), and in the latter case, it was obtained with the correction of bacterial increase by the method of Frost (1972). The bacterial number was counted by the acridine orange direct count method (Hobbie et al. 1977).

Results

From both the SEM photograph (Fig. 1) and the light microscope (Nomarski) photograph (Fig. 2), principal features of fine structure of the filtering comb in *D. longispina* can be understood in consideration of the fact that all of setules in the SEM images are noticeably shrinked or deformed probably through the preparation process for SEM. Two rows of setules project from one seta toward both sides with some angles (Figs. 1 and 2a) and all setules project in

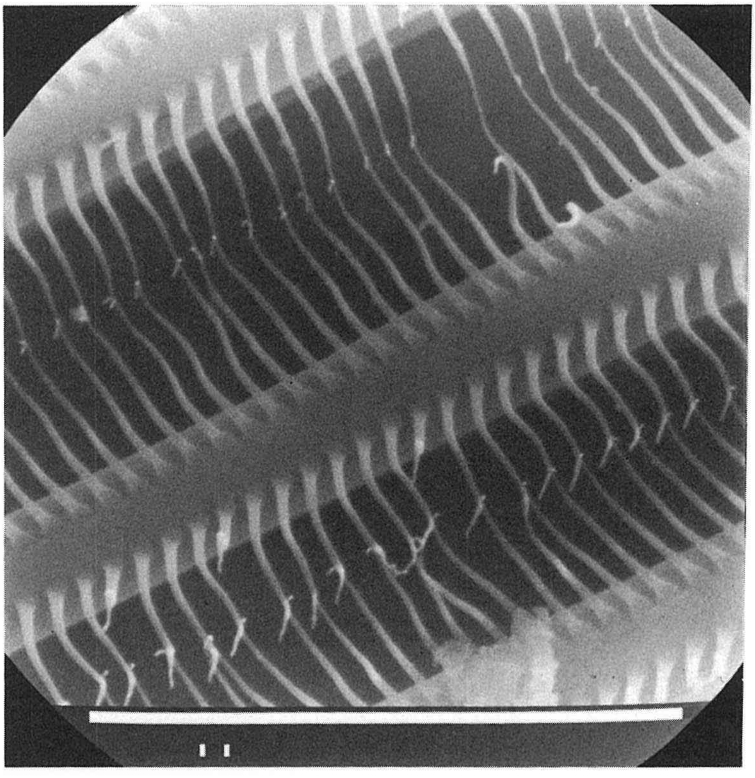

Fig. 1. An SEM photograph of a filtering comb of *D. longispina* in Lake Biwa. Comparing with the light microscopic photographs (Fig. 2), shrinkage and deformation of setules are found. Note the three-dimentional structure of the filtering comb. Length of bar in Figure is 10 μm.

parallel (Fig. 2b). The length of setule is almost the same as intersetal distance (Fig. 2b). The setules projecting from adjacent setae cross at the intersetal spaces (Fig. 2a). Hence there is no "intersetal gap" (Kořínek and Macháček 1980).

Diameter of setules (ϕ in μm), intersetulal distance (D in μm) and mesh-size (M in μm) increased with the increase of body length (L in mm) (Fig. 3). These parameters could be regressed as follows; $\phi=0.130\ L^{0.40}$ (r=0.78; n=14), $D=0.402\ L^{0.40}$ (r=0.88; n=14) and $M=0.270\ L^{0.40}$ (r=0.82; n=14). The range of mesh-size was from 0.17 to 0.36 μm. The maximum mesh-size was estimated to be about 0.4 μm in adult *Daphnia* of maximum size, being approximately 1.4 mm in Lake Biwa.

The feeding experiment revealed that *Daphnia* ingested natural bacteria ranging from 4.1×10^5 to 4.5×10^6 cells·ml^{-1} (Table 1). The range of the filtering rate was 0.3–0.9 ml·anim^{-1}·h^{-1} without significant dependency on the bacterial densities and the mean value for all the experimental series was 0.57 ± 0.09 ($\pm$S.E.; n=7) ml·anim^{-1}·h^{-1}.

Discussion

The filtering mesh-sizes of *D. longispina* in Lake Biwa were below 0.4 μm (Fig. 3) and most

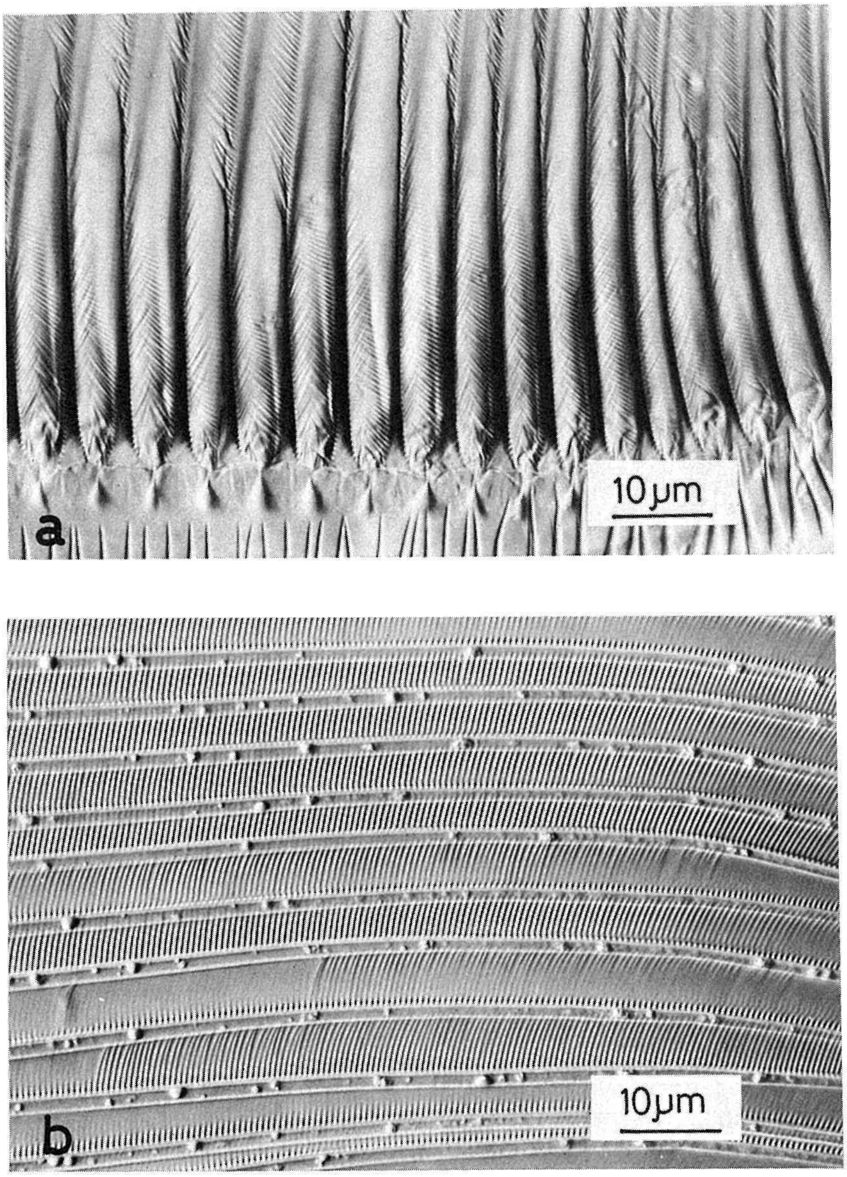

Fig. 2. Light microscopic (Nomarski) photographs of a filtering comb of *D. longispina* in Lake Biwa. (a) Basal part of the filtering comb. Note that two rows of setules project from one seta and setules from two adjacent setae cross at the intersetal spaces. (b) Rows of setules on one side are pressed by a cover slip. Note the setules projecting in parallel and reaching from one seta to adjacent one.

parts (more than 90%) of bacteria consisted of larger cells than 0.4 µm in this lake (Nagata unpublished). If *Daphnia*'s feeding mechanism is completly based on sieving process, therefore, it is suggested that this *Daphnia* would be able to feed efficiently on natural free-living bacteria in the lake throughout its whole life stages.

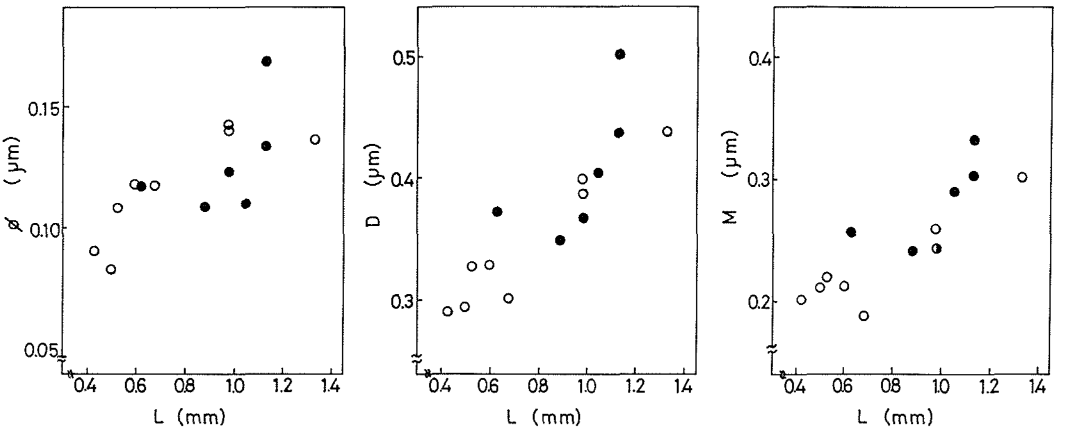

Fig. 3. Diameter of setule (ϕ), intersetulal distance (D) and mesh-size ($M=D-\phi$) in relation to body size (L) of *D. longispina* sampled at the pelagic area of the north basin of Lake Biwa on 13 October 1983 (○) and 10 January 1984 (●).

Table 1. The filtering rates of *D. longispina* on natural bacteria in Lake Biwa.

Bacterial density ($\times 10^6$ cells · ml^{-1})	Filtering rate (ml · $anim^{-1}$ · h^{-1})
4.51	0.7, 0.6
2.20	0.4, 0.9
1.20	0.8
0.41	0.3, 0.3

According to the classification of filter feeding Cladocera based on filter mesh-size (Geller and Müller 1981), *D. longispina* in Lake Biwa can be grouped into "high efficiency bacteria feeders" which have fine filter meshes from 0.24 to 0.64 μm. On the other hand, *Daphnia hyalina* and *D. galeata* in Lake Constance, both of which are nearly relative species to *D. longispina*, have the wider filter meshes (0.56–1.8 μm about *D. hyalina* and 0.32–1.0 μm about *D. galeata*) and so are classified into "low efficiency bacteria feeders" (Geller and Müller 1981). Such differences in the mesh-size among these species seem to be attributed not only to the difference in body size, that is, maximum body length of *D. longispina* (ca. 1.4 mm) in Lake Biwa is smaller than that of *D. hyalina* and *D. galeata* (ca. 2.1 mm) in Lake Constance, but also to the difference in relative growth of the mesh-size to body length. The rate of increase in the intersetulal distance relative to body length was 1.33 as an exponential value for *D. hyalina* and about half as great for *D. galeata* (Geller and Müller 1981) but 0.40 for *D. longispina* in Lake Biwa.

The filtering rates of *D. longispina* (L, 1.0–1.3 mm) on natural bacteria alone, which density ranged from 4.1×10^5 to 4.5×10^6 cells·ml^{-1}, was in the range of 0.3–0.9 ml·$anim^{-1}$·h^{-1} (Table 1). Fed on natural phytoplankton in Lake Biwa, the filtering rate of this *Daphnia* (L, 1.0–1.3 mm) was in the range of 0.5–1.1 ml·$anim^{-1}$·h^{-1} (at 15°C) for the <25 μm fraction of the phytoplankton (Narita unpublished), and also 0.3–0.6 ml·$anim^{-1}$·h^{-1} in summer (at

27–28°C) and about 0.2 ml·anim^{-1}·h^{-1} in winter (at 11–14°C) for the <10 μm algal fraction in a phytoplankton assemblege (Okamoto 1984). This supports the above-mentioned presumption that *D. longispina* is a "high efficiency bacteria feeder".

Although it has been hypothesized that "high efficiency bacteria feeders" appear only in eutrophic lakes (Geller and Müller 1981), they actually inhabit as a predominant zooplankter in a mesotrophic basin of Lake Biwa. Hence, a *Daphnia*-bacterial trophic link should be considered to have significant ecological implications not only in eutrophic lakes but also in mesotrophic lakes such as the north basin of Lake Biwa. For example, epilimnetic bacterial density ($3–7 \times 10^6$ cells·ml^{-1}, corresponding to approximately 50–100 μgC·l^{-1} in biomass) in this basin during warm seasons (Nagata 1984 and unpublished) imply that bacteria is possibly a quantitatively significant food source for *D. longispina*. As further problems to be solved, it is vital to clarify the bacterial contribution to the total food requirement of *Daphnia* as well as the feeding effects of *Daphnia* on bacterial population dynamics in Lake Biwa.

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References

Frost, B. W. (1972) Effects of size and concentrations of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnol. Oceanogr. 17: 805–815.

Geller, W. and H. Müller (1981) The filtration apparatus of Cladocera: filter mesh-sizes and their implications on food selectivity. Oecologia 49: 316–321.

Gophen, M., B. Z. Cavari and T. Berman (1974) Zooplankton feeding on differentially labelled algae and bacteria. Nature 247: 393–394.

Hobbie, J. E., R. J. Daley and S. Jasper (1977) Use of nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. 33: 1225–1228.

Kořínek, V. and J. Mahcáček (1980) Filtering structures of Cladocera and their ecological significance I. *Daphnia pulicaria*. Věst. čs. Společ. zool. 44: 213–218.

Nagata, T. (1984) Bacterioplankton in Lake Biwa: annual fluctuations of bacterial numbers and their possible relationship with environmental variables. Jap. J. Limnol. 45: 126–133.

Okamoto, K. (1984). Size-selective feeding of *Daphnia longispina* and *Eodiaptomus japonicus* on a natural phytoplankton assemblage with the fractionizing method. Mem. Fac. Sic., Kyoto Univ. Ser. Biol. 9: 23–40.

Peterson, B. L., J. E. Hobbie and J. F. Haney (1978) *Daphnia* grazing on natural bacteria. Limnol. Oceanogr. 23: 1039–1044.

Tezuka, Y. (1971) Feeding of *Daphnia* on planktonic bacteria. Jap. J. Ecol. 21: 127–134.