Size-selective Feeding of *Daphnia longispina hyalina* and *Eodiaptomus japonicus* on a Natural Phytoplankton Assemblage with the Fractionizing Method*

By

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Abstract. The feeding and filtering rates of *Daphnia longispina hyalina* and *Eodiaptomus japonicus*, dominant herbivorous zooplankters in the pelagic area of Lake Biwa, on the natural phytoplankton assemblage were examined five times from summer to winter in the laboratory using the fractionizing method. The assemblage was divided into four different sized fractions (150–70, 70–25, 25–10 and <10 μm), which were separately labelled with ^{14}C. The feeding and filtering rates of some size classes of the zooplankters were determined for each labelled fraction under the coexistence of the other non-labelled three fractions. The food size-selection was also examined with the Ivlev's index and filtering efficiency. The availability of the fractionizing method and the usefulness of the filtering efficiency as a selectivity index were discussed and appraised.

1. Introduction

Knowledge on zooplankton grazing is essential to understand not only a flow of energy and matter in a pelagic ecosystem but also changes in the abundance and the species composition of phytoplankton. Planktonic algae in a natural water vary in size, shape, systematic state and chemical qualities. When measuring the grazing rates (that is, feeding and filtering rates) of zooplankters on a natural phytoplankton assemblage, therefore, selective feeding manners of zooplankters should be taken into account. On this account, it would be most ideal that the feeding and filtering rates are determined on each planktonic alga composing the assemblage. But, that is impossible in fact today and probably even in the near future. How to measure those rates is a vital question for the study of grazing on natural phytoplankton.

There have been a few studies on the selective feeding of cladocerans and calanoid copepods, principal herbivores in freshwater bodies, on a natural phytoplankton assemblage (McQUEEM 1970; BERMAN & RICHMAN 1974; BOGDAN & McNAUGHT 1975; GLIWICZ 1977; BOWERS 1980; VANDERPLOEG 1981). Among these, either of the following three methods has been used to estimate the feeding and filtering rates; 1) measuring the change of algal concentration in cell density or chlorophyll *a* amount during a given feeding period, 2) adding certain artificial particles with various sizes as tracer into a natural phytoplankton assemblage, 3) dividing a natural phytoplankton assemblage into two size fractions of nanno- and netplankton parts, and

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then labelling them separately with ^{14}C. Nevertheless, both of the first and second methods have some methodological problems unsolved. In the first method, food condition changes considerably both in quantity and quality during the experiment because of the necessity of a long feeding period. For the second method, it is doubtful whether the artificial particles as tracer can be selected and ingested by zooplankton as well as natural phytoplankters. Accordingly, the principles for the measurement of feeding activities of zooplankton on a natural phytoplankton assemblage can be considered at least to use directly natural phytoplankton as food and to adopt a feeding period as short as possible.

The third method, which was proposed by BOGDAN and McNAUGHT (1975), seems to satisfy the above principles. But, the fractionation of phytoplankton into only two size groups does not reflect adequately a complex size-structure of phytoplankton assemblage. Therefore, the present study offers an improved and advanced method in which a natural phytoplankton assemblage is fractionized into four size groups. This method enables 'to measure the feeding and filtering rates of zooplankton on a given fraction, which is labelled with ^{14}C, in the presence of other size fractions which are non-labelled.

With this method, the feeding and filtering rates of two herbivorous zooplankters (*Daphnia longispina hyalina* and *Eodiaptomus japonicus*) on a natural phytoplankton assemblage of Lake Biwa were examined five times under the laboratory condition from August, 1975 to February, 1976. From these results, the availability of this method for elucidating size-selective feeding activities of the zooplankters is demonstrated. Filtering efficiency and its curve pattern are also proposed as a useful index of the food-selection, and then some characteristics of the preferential feeding of these zooplankters are discussed in relation to algal food conditions.

2. Materials and Methods

2–1. The procedure of the feeding experiment.

Both of the zooplankters and the phytoplankton as food for the experiments were collected at a fixed station (44 m deep) in the main basin of Lake Biwa. The zooplankters collected by vertical tows of a net (100 μm aperture) within the epilimnion were kept in a large container (45 liter in volume) filled with surface lake water, and transported to the laboratory. Water samples for the phytoplankton as food were always taken from the 5 m layer, which is usually characterized by high biomass and productivity of phytoplankton (NAKANISHI 1976).

The phytoplankton assemblage in the lake water was divided into four size fractions of 150–70 μm, 70–25 μm, 25–10 μm and smaller than 10 μm with four kinds of screens (150 μm, 70 μm, 25 μm and 10 μm aperture) (Fig. 1). The fraction larger than 150 μm in size was discarded to eliminate zooplankton. This fraction was quite negligible in quantity of chlorophyll a in comparison with the whole assemblage and also considered to be hardly utilized by the zooplankters as food.

Each of the three fractions of 150–70 μm, 70–25 μm and 25–10 μm was concentrated to 400 ml from 80 liters of the lake water, while the filtrate was used as the smallest fraction ($<$10 μm). Each fraction was separated into two groups, the labelled group with ^{14}C-sodium bicarbonate (NaH^{14}CO$_3$) and the non-labelled one. The former group of the three fractions (150–70 μm, 70–25 μm and 25–10 μm) was labelled with 10–20 μCi of ^{14}C as NaH^{14}CO$_3$. The

Lake Water
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Size Fractionation of Algae
with Screens (150, 70, 25 & 10 μm aperture)

> 150 μm	150 - 70 μm	150 - 70 μm
	70 - 25 μm	70 - 25 μm
	25 - 10 μm	25 - 10 μm
	< 10 μm	< 10 μm

Discarded ^{14}C - Labelled   Non - Labelled
|
Recombination
|
(Algal Mixture)   (1)   (2)   (3)   (4)
150 - 70 μm
70 - 25 μm
25 - 10 μm
< 10 μm   ← Labelled Fraction
                                        (Shaded Part)
|
Fed to Zooplankters

Collecting of Zooplankters
|
Killing and Cleaning
|
Size Classification of a Given Species
|
NCS - Treatment
|
Adding of Gupta's Fluor
|
Counting of Radioactivity
with a Scintillation Counter

Fig. 1. The experimental procedure of the algal size fractionation, food preparation and treatment processes after feeding.

^{14}C solution with 30–35 μCi was added to 2 liters of the smallest fraction. The labelled and non-labelled groups were exposed to approximately 20 Klux for 18 hours, except the February experiment (3 hours). During the incubation, the suspension of each group was stirred to avoid sedimentation of particles.

After the incubation, one labelled fraction was mixed with three other non-labelled fractions so as to be 2 liters in total, in which the phytoplankton density of each fraction was adjusted to the original one before the fractionation (Fig. 1). Four kinds of the algal mixture were made up and provided separately for the zooplankters as food. Consequently, incorporated radioactivities into the zooplankters feeding on one of these mixtures represented their ingestion on a given size-fraction alone among the total phytoplankton assemblage of four fractions.

In the feeding experiment, about 200 animals of various sizes of the zooplankters were put into a feeding chamber (tri-conical flask) where 2 liters of the algal mixture with some labelled fraction had been already prepared, and allowed to feed for 15 and 30 minutes under the dim light condition. The water temperature of the feeding chamber was kept to be nearly equal to that in the field where the zooplankters had been collected. After feeding, the zooplankters were trapped on a net (150 μm aperture) and killed immediately by dipping the animals into boiled water. Then, they were rinsed five times with filtered lake water to remove excess phytoplankton attached on their bodies and dipped into 0.001N HCl solution to remove residual

^{14}C adhering to the surface of their bodies. The residue of the HCl solution on the bodies was rinsed out with deionized water to avoid chemical quenching at the measurement of radioactivity. Both of *Daphnia* and *Eodiaptomus* were sorted into size classes at an interval of 0.2 mm in length under a dissecting microscope. The length was measured with a micrometer from the center of the eye to the base of spine for *Daphnia* and from it to the end of the 6th thorasic segment for *Eodiaptomus*. Each size class of the respective species was put into a vial, to which 0.5 ml of tissue solubilizer (NCS) was added. The vials were incubated at 42°C for 20 hours. After the incubation, 10 ml of a fluor composed of 4 g PPO, 100 mg POPOP, 80 g crystallized naphthalene, 50 ml P-dioxane made up to 1 liter with scintillation grade toluen was added to each vial and then radioactivies were measured with a liquid scintillation counter (Aloka, LSC 502).

To determine the radioactivities of phytoplankton used for the feeding experiment, the respective water samples with four kinds of labelled algal fractions were filtered on an HA Millipore filter (0.45 μm). Radioactivities of labelled algae in Bray's fluor (Bray 1960) were measured with a liquid scintillation counter.

The particulate organic carbon (POC) contents of non-labelled fractions were measured with a CHN analyzer (YANAKO, MT-2), and were used in the calculation of feeding rates for the respective labelled fractions. Also, the phytoplankton species composition except the February samples was examined for the fractions preserved in approximately 0.1% of folmalin.

2-2. The calculation of the feeding and filtering rates.

Feeding and filtering rates for each size class of *Daphnia* and *Eodiaptomus* were calculated on each algal fraction among the whole assemblage as follows;

$$\text{Feeding rate (carbon } \mu g \cdot animal^{-1} \cdot hr^{-1}) = I \times \frac{C}{P} \times 60$$

$$\text{Filtering rate (ml} \cdot animal^{-1} \cdot hr^{-1}) = \frac{I}{P} \times 60000$$

where, I is dpm of the incorporated ^{14}C into a zooplankter in one minute (animal^{-1}·min.$^{-1}$), P, dpm of a certain labelled fraction of algae of 1 liter, and C, carbon content in the same non-labelled fraction of 1 liter (μg).

For calculation of the feeding and filtering rates on the smallest fraction, the measured values of the incorporated carbon amounts during the first 15 minutes-feeding period were used. For other fractions, considering the possible occurrence of rejection by the zooplankters between 15 and 30 minutes in the feeding time course, the measured values of the incorporated carbon amounts during the first 15 minutes-feeding period were revised as follows;

$$\text{A (an estimated value)} = B \times \frac{C}{D} \quad \text{(See Fig. 3)}$$

where A is an estimated incorporation in carbon amount at the feeding time of 15 minutes,

  B, a measured incorporation in carbon amount at the feeding time of 30 minutes,

  C, a measured incorporation in carbon amount at the feeding time of 15 minutes for the smallest fraction, and

  D, a measured incorporation in carbon amount at the feeding time of 30 minutes for the

smallest fraction.

For the revision, it was assumed that every fraction of the ingested food would pass through the intestine of the zooplankters at the same speed. The necessity and validity of the revision will be discussed afterwards.

Size-selectivity of zooplankters for the four different sized algal fractions was examined with the Ivlev's index and filtering efficiency. Filtering efficiency was calculated as follows;

$$f \text{ (filtering efficiency)} = \text{filtering rate on a given fraction/the maximum filtering rate among the four fractions.}$$

3. Results and Discussion

3–1. The quantitative and qualitative characteristics of the four algal fractions.

The POC contents and algal species compositions of the four fractions were largely different among the five experiments carried out from summer to winter (Tables 1 & 2). The total food concentration was high in summer, but low in autumn and winter. The food concentrations of the largest and second largest fractions varied seasonally. Their high abundance found in summer were due to a propagation of large green algae such as *Staurastrum dorsidentiferum* and *Pediastrum Biwae*. The 25–10 μm and the smallest fractions showed a relatively small variation in food concentration among the experiments. The 25–10 μm fraction was always lowest in quantity among the four fractions in every experiment. Seasonal change in food amount was much more remarkable in the largest and second largest fractions than in the other smaller fractions. Accordingly, the changes in the first two fractions caused mainly the observed large variation in the total food amount.

The algal species composition in the largest fraction differed clearly between the warmer and the colder seasons (Table 2). This fraction consisted mainly of large green algae with a complex or long shape, or being colonial, such as *S. dorsidentiferum*, *S. tohopekarigense*, *Closterium aciculare* and *P. Biwae*, in August and September. The most part of it in November and December consisted of a chained diatom, *Melosira solida*. The second largest fraction was composed of green algae such as *Coelastrum* spp., *Sphaerocystis shroeteri* and *Oocystis* spp., to some extent, in addition to some species of the above-mentioned large sized algae. This fraction was most diverse in species composition among the four fractions. In the 25–10 μm fraction, green algae with large size were scarce. The main components were unicellular and small sized algae such as *Oocystis* or a short piece of broken colonial algae such as *Melosira* and *Sphaerocystis*. The

Table 1. The food concentrations of the four algal fractions in each experiment. (μgC·1^{-1})

Fraction size	Aug. 12 1975	Sept. 19	Nov. 19	Dec. 21	Feb. 19 1976
150–70 μm	222.6	190.3	122.0	78.4	34.9
70–25 μm	35.9	361.7	70.4	99.0	34.9
25–10 μm	17.9	25.3	19.3	37.4	21.8
<10 μm	140.0	225.1	117.6	104.7	162.1
Total	416.4	802.4	329.3	319.5	253.7

Table 2. The main species composition and the abundance of phytoplankton in the four fractions used as food

Date	Aug. 12				Sept. 18				Nov. 29				Dec. 21			
Fraction size (µm)	150–70	70–25	25–10	<10	150–70	70–25	25–10	<10	150–70	70–25	25–10	<10	150–70	70–25	25–10	<10
Staurastrum dorsidentiferum	++	+	+	r	++	++	r		++	+			+	+		
S. tohopekaligense	+				+				+				r			
Closterium aciculare	+	r			r		r		+	r			+	+	r	
Spondylosium moniliforme	r	++			+	+	r		r				r			
Xanthidium hastiferum							r		r				r			
Pediastrum Biwae	++	++	+		++	++	r			+			+	r		
Coelastrum spp.		+	r	r		r	r	r		r	r		r	r	r	
Oocystis sp.		+	++	r		+	+	r		+	+		r	r	r	r
Spaerocystis schroeteri		r		r			r		+	+	+			r	+	r
Melosira solida		r			+	+	+	r	++	++	++	r	++	++	++	r
M. italica		r	r		r	r			r	r					+	
M. granulata	+	r	r		+	+	r		r	r						
Aphanocapsa sp.		+	+													
Anabaena sp.		r	r													
ultraplankton				**				**				**				**

Symbols of phytoplankton density (cells·liter^{-1})   ++ : >10^4,   + : 10^3–10^4,   r : <10^3
The smallest fraction (<10 µm) is shown in a relative abundance as follows;   ** : abundant,   r : rare

smallest fraction consisted mainly of ultraplankton which could not be identified.   Its algal species composition was clearly different from those of the three other fractions.

    Among these three fractions, a part of algal components in a fraction was usually common to those in one or two other fractions in every experiment.   The degree of similarity in the algal species composition between these fractions was examined with Morisita's index of community similarity, C_λ' (Morisita 1971) (Table 3).   In August and September when large green algae such as *S. dorsidentiferum* and *P. Biwae* were propagated, the index value was high between the largest and second largest fractions, while low in the comparison of both fractions with the 25–10 µm fraction.   It suggests that the 25–10 µm fraction was considerably different in the algal species composition from the two other larger fractions.   In November and December, however, the index values between any fractions were near to 1.0, showing that the species composition in any of the three fractions was similar with each other.   This is due to

Table 3. Comparisons of the similarity of the algal species composition among the three food fractions of 150–70 µm (A), 70–25 µm (B) and 25–10 µm (C), by means of the Morisita's measure of community similarity, C_λ'.

Sets of comparison	Experimental months			
	August	September	November	December
A to B	0.712	0.974	0.952	0.861
B to C	0.521	0.260	0.892	0.612
C to A	0.303	0.244	0.994	0.967

    The value of C_λ' (Morisita 1971) is about 1 when the two samples belong to same community and is zero when no common species is found between them.

the fact that *M. solida* was abundantly included even in the 25–10 μm fraction. Supposedly, this alga is easily broken in pieces of one or a few cells.

Thus, net-fractionizing of a phytoplankton assemblage is of a little avail for discriminating a rough structure of the assemblage in accordance with the algal species composition, though the availability varies depending on the difference in the characteristics of dominant species in the phytoplankton assemblage.

In the present study, the largest fraction (150–70 μm) is necessary as the representative of net phytoplankton, since a plankton net of approximately 70 μm aperture in mesh size is usually used for a plankton sampling in Lake Biwa. The lower border size of 25 μm in the second largest fraction (70–25 μm) corresponds to the estimated maximum size of edible food particles for a daphnid adult (1.0 mm long) (BURNS 1968a). The size of 10 μm as the border between the 25–10 μm fraction and the smallest one is arbitrarily adopted to distinguish between nanno-phytoplankton and ultraplankton. This size was valid, judging from the fact that the algal species composition was distinctly different between the 25–10 μm fraction and the smallest one. The former fraction was always least in food concentration, resulting in the lowest availability for zooplankton. Therefore, it may not be necessary to set this size-group as a food fraction in the phytoplankton assemblage of Lake Biwa.

3–2. Revision of the carbon incorporation influenced by zooplankton rejection.

When feeding and filtering rates of zooplankton are measured using radioisotope-labelled algae as tracer of food, they should be determined based on the incorporated radioactivities into the animals before the beginning of defecation. The carbon incorporation by *Daphnia* and *Eodiaptomus* in the present study was not proportional to the feeding time (30 minutes), since most of lines are out of straight in any of the four fractions, especially the largest and the second largest ones (Fig. 2). A cause for the decrease in the incorporation amount during the latter 15 minutes seems to be the loss of ingested food due to defecation, which had occurred between 15 and 30 minutes in the feeding time course (BELL & WARD 1970; GELLER 1975). However, the lines of the three larger fractions (150–70 μm, 70–25 μm and 25–10 μm) are doglegged more steeply than those of the smallest fraction. It suggests that there might be other causes for such break of the increase in the carbon incorporation, in addition to defecation.

As possible causes, the following two can be conceived: (1) a large part of the food fraction collected by filtration was rejected from the thoracic chamber or food groove of a zooplankter before ingested, (2) after ingested, the larger fractions were less efficiently assimilated than the smallest fraction during a passing through the intestine. The latter is insufficient as the main cause on all cases of such inflection, even if it were in action. Because, some observed phenomena that the incorporated carbon amount all over the feeding time of 30 minutes was less than that incorporated for the first 15 minutes cannot be theoretically explained by the latter cause.

As to the former cause, some observations have been reported. Daphnids reject unwanted food material and excess amount of even favourable food particles from the thoracic chamber by the abdominal movements and from the food groove by the maxillules (McMAHON & RIGLER 1963; BURNS 1968b). They also prevent the entrance of extralarge algae or special kinds of blue-green algae by narrowing the carapace gap (GLIWICZ & SIEDLER 1980). GLIWICZ and
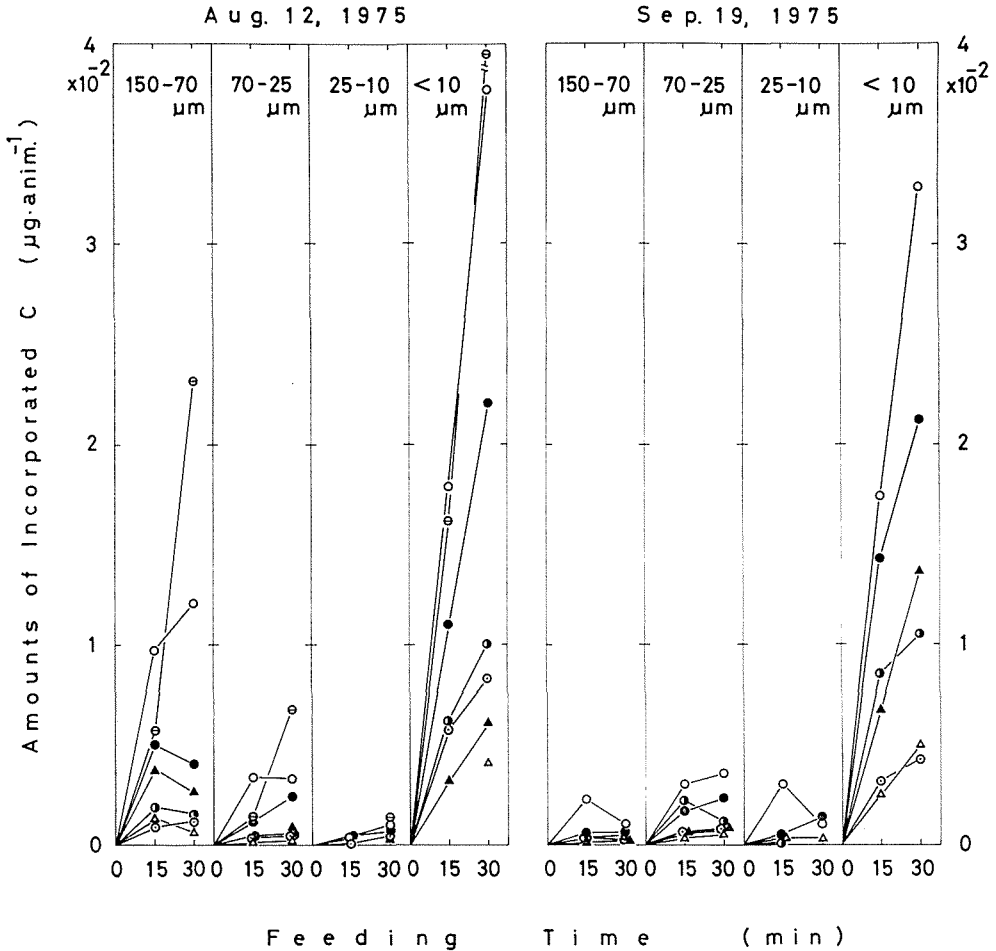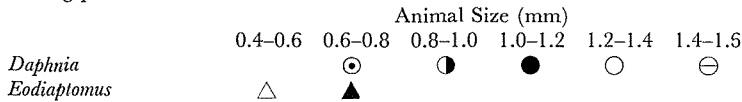
Fig. 2.   Incorporated carbon amounts of the four fractions by some animal size classes of zooplankters during a feeding period of 15 and 30 minutes.

	Animal Size (mm)					
	0.4–0.6	0.6–0.8	0.8–1.0	1.0–1.2	1.2–1.4	1.4–1.5
Daphnia		⊙	◖	●	○	⊖
Eodiaptomus	△	▲				

SIEDLER (1980) suggested that the rejectory behaviour would be induced when a natural phytoplankton assemblage is dominated by net phytoplankton like diatom or large green algae. This should be taken a special note in the present study, in which the largest and second largest fractions of the natural lake phytoplankton were dominated by large green algae or diatoms (cf. Table 2).   The period of 15 minutes in the present study corresponds approximately to the time of the first occurrence of the rejection or the narrowing behaviour (MCMAHON & RIGLER 1963; GLIWICZ & SIEDLER 1980).   As for calanoids copepods, the rejectory response to unwanted particles with the second maxillary setae has been found (ALCARAZ *et al.* 1980; DONAGHAY 1980; FRIEDMAN 1980).

Hence, it is much conceivable that frequent rejection against the larger fractions occurred

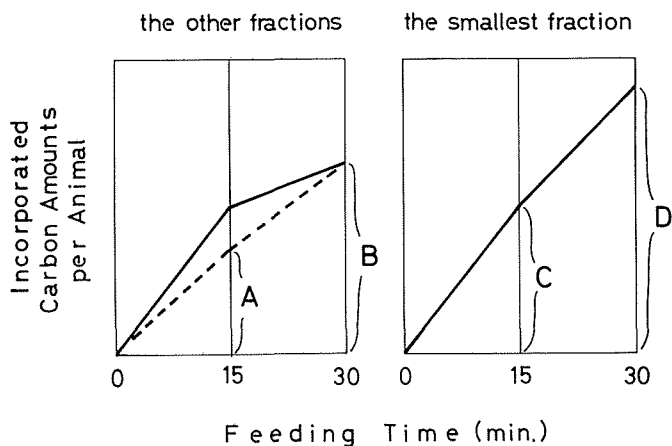the other fractions   the smallest fraction

Fig. 3.   A schema for revising of incorporation rates on the largest, second largest and 25–10 μm fractions during the first 15 minutes.
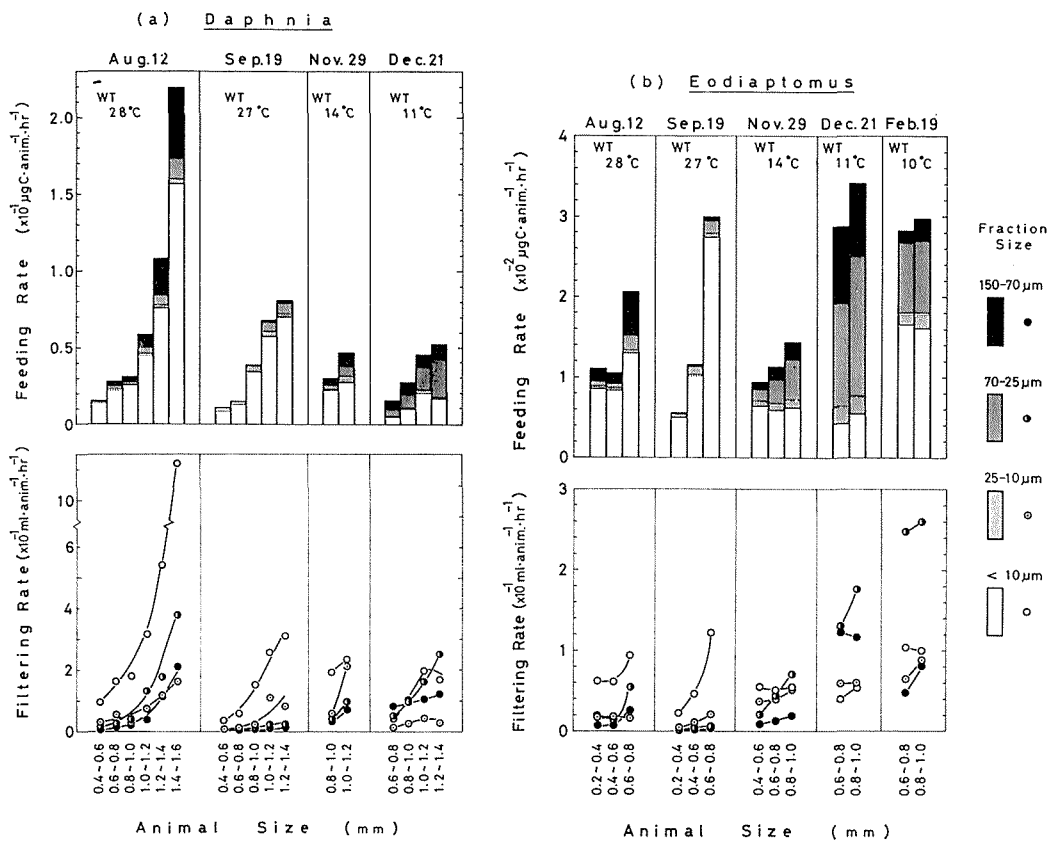

Fig. 4.   The feeding and filtering rates of *Daphnia* (a) and *Eodiaptomus* (b) on the four fractions, in relation to animal size (length).

between 15 and 30 minutes in the present experiments, resulting in the decrease in the ingestion on those fractions and then in the inflection of the lines at 15 minutes of the feeding time in Fig. 2. The food collected before the initiation of the rejection includes a portion which should be rejected later on. If the feeding rates on those large fractions are directly calculated based on the radioactivies incorporated during the first 15 minutes, they would be overestimated. It is necessary to revise the directly calculated carbon incorporation and to estimate the carbon amount which would be actually ingested. The process of revision adopted here (Fig. 3) is based on the assumption that every fraction of the ingested food would pass through the intestine of the zooplankter at the same speed. There has been no report on the difference in passing speeds of various food particles through the intestine.

3–3.   Variations of the feeding and filtering rates on the four algal fractions.

All of the size classes of both *D. longispina* and *E. japonicus* fed all algal fractions of four sizes at a time. The feeding and filtering rates increased with increasing of the animal size in both zooplankters, but they were not proportional either to temperature or to food concentration among the five experiments carried out under the different thermal and nutritional conditions (Fig. 4). Comparing the results between August and September, the feeding rates of both zooplankters did not increase in proportion to the increase in food amount. *Daphnia* in November and *Eodiaptomus* in December and February suggested that the lower temperature did not lead necessarily to the decrease in the feeding or filtering rates on natural phytoplankton. Thus, the feeding activity of zooplankton on a natural phytoplankton assemblage cannot be
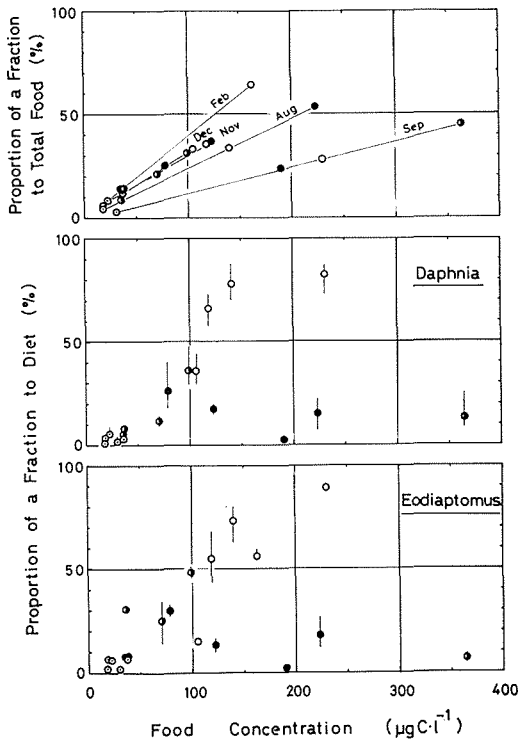

Fig. 5.   Relationships between the food concentrations of the four fractions and their proportions in diet. A plot means an average proportion among animal size classes and a vertical bar represents the range.

● : 150–70 μm   ◑ : 70–25 μm   ◉ : 25–10 μm
○ : <10 μm

understood as simply as that on a cultured alga which can be explained well in relation to temperature and food concentration (BURNS 1969; GELLER 1975).

Daphnia feeding was characterized by a high contribution of the smallest fraction to the diet, 67.8% on the average (Fig. 5), even when the largest and second largest fractions had high percentages in the total food amount. It suggests the preferential feeding of *Daphnia* to the smallest fraction. In *Eodiaptomus*, also, the average contribution of the smallest fraction to the diet was 61.0%, indicating that this fraction was the most important food resource among the four algal fractions for the copepod as well as for the daphnid. In December, however, the contribution of the largest and second largest fractions increased, suggesting a change in the size-preferential feeding by the zooplankters.

Such preferential feeding can be also implied by the differences in filtering rates among the four fractions within an experiment (Fig. 4). In *Daphnia*, the filtering rate on the smallest fraction was higher than those on the other fractions in any experiments except in December, indicating efficient filtration, or retention, of small algal particles. In *Eodiaptomus*, the filtering rate was higher on the smallest fraction than on the other ones in August and September, but similar among the fractions in November, and then turned to be highest on either the largest or the second largest fraction in December and February. The change in the size of fraction
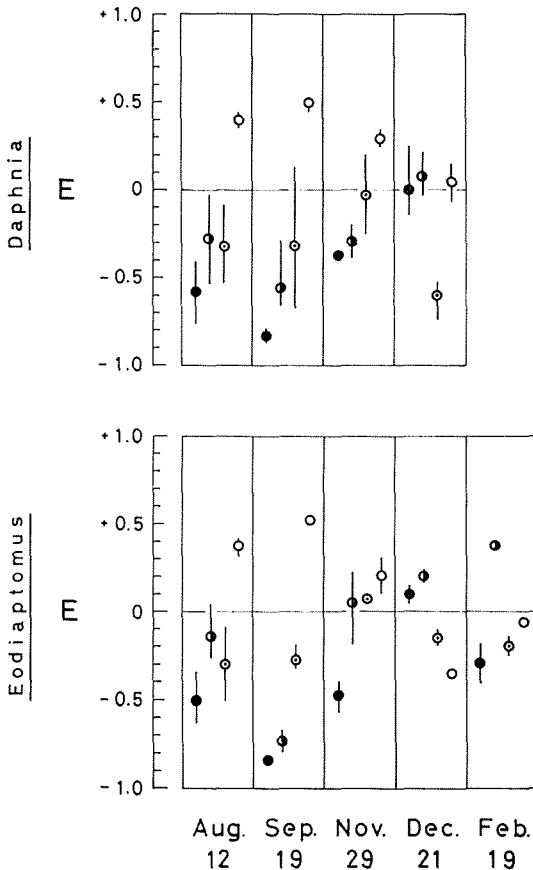

Fig. 6. Seasonal changes in food size-selectivity (IVLEV's index) in *Daphnia* and *Eodiaptomus*.
● : the largest fraction (150–70 μm)
◑ : the second largest fraction (70–25 μm)
⊙ : the 25–10 μm fraction
○ : the smallest fraction (<10 μm)

filtered most efficiently suggests the change in the size-selective feeding by the copepod.

3–4.   Filtering efficiency and its curve pattern as an index of size-selectivity.

Figure 6 shows the changes of IVLEV's electivity indices for the four food fractions.   It indicates that *Daphnia* selected the smallest fraction in August, September and November, while such selection disappeared in December.   *Eodiaptomus* showed different changes in the electivity from *Daphnia*. The exclusive selection for the smallest fraction in August and September was weakened in November, and then followed by the distinct selection for the largest and second largest fractions in December and for the latter one in February.

As the filter-feeding of the zooplankters is a kind of mechanical sieving through their setae, the difference in filtering rates among the four fractions in one experiment represents the difference in the efficiency of filtration (or retention), that is, the preference to the food fraction.   Figure 7 shows the filtering efficiency (f) curve among the four fractions.   In *Daphnia*, as the efficiency of the smallest fraction surpassed the others (because the filtering rate on the
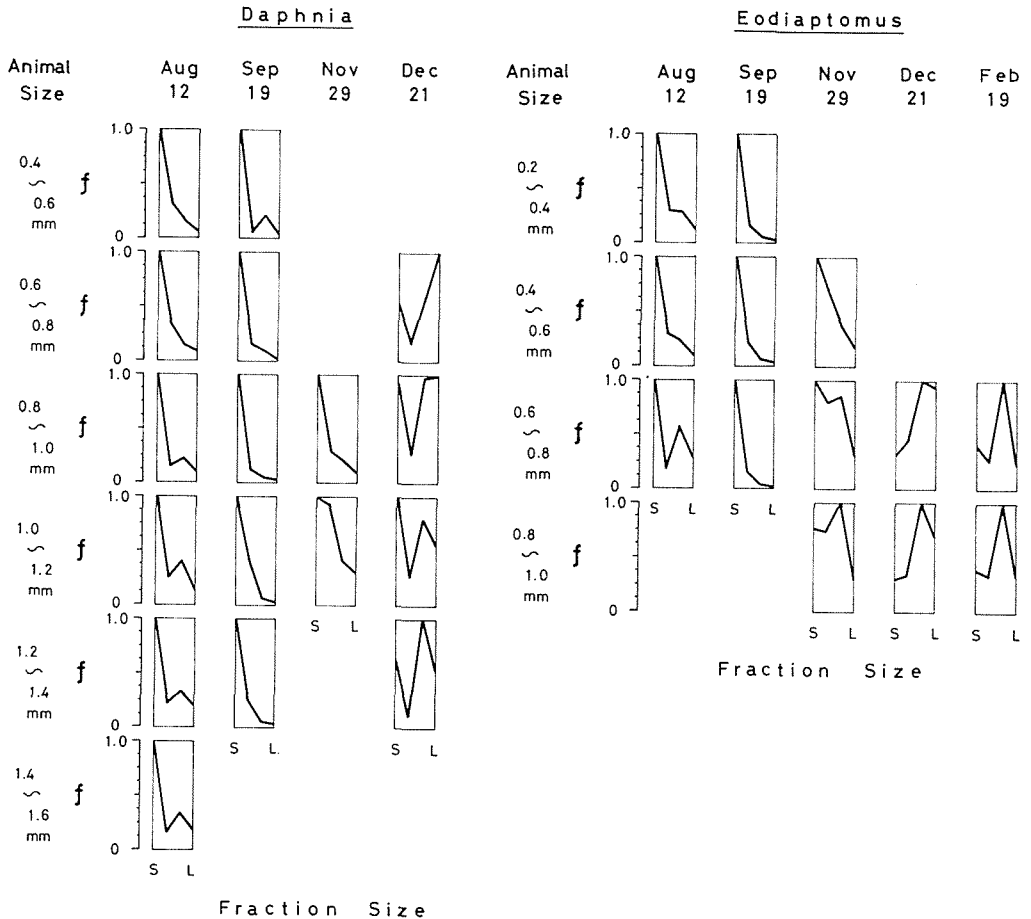

Fig. 7.   Seasonal changes in the filtering efficiency (f) curve in some animal size classes of *Daphnia* and *Eodiaptomus*. Fraction size in the abscissa is arranged in order of the smallest (S), the 25–10 μm, the second largest and the largest (L) fractions from left to right side.

smallest fraction surpassed) in all animal sizes in August, September and November, the pattern of the *f* curve formed L-shape representing the selection for the smallest fraction. In December, the fraction with the maximum efficiency was different among the animal size classes. However, the efficiency was not largely different among the fractions except the 25–10 μm fraction, suggesting that *Daphnia* might feed nonselectively regarding to the fraction size.

In *Eodiaptomus*, the highest efficiency was found in the smallest fraction in August and September, resulting in L-shape, while in the second largest fraction in February, resulting in convex shape. In November, however, the efficiencies were even among the three fractions except the largest one, resulting in deformed L-shape. In December the efficiencies were high in the two larger fractions and low in the two smaller ones, showing reverse L- or convex shape.

The *f* curve patterns in one experiment were almost similar among the size classes in the respective zooplankters. It suggests that the inclination of the food-size selection was similar in any size class of every zooplankter in a certain month. Thus, the filtering efficiency and its curve pattern are useful as an indicator of selective feeding.

The IVELEV's electivity index, derived from a relationship of percent to percent between food concentration and diet, is apt to be strongly affected even by a small change either in food concentration or in feeding rate. Thus, a value of the IVELEV's index for one fraction is dependent on the values for other fractions. This index is less informative on selective mechanisms (e.g. rejection, pass-out or raptorial seizing) in the filter-feeding process of a zooplankter, since it is calculated without consideration on that process.

The filtering efficiency, based on the filtering process, is completely different for conception from the IVELEV's index. This efficiency represents the degree of captured amount to the whole amount of a fraction existing in filtered water mass, which is given as the maximum filtering
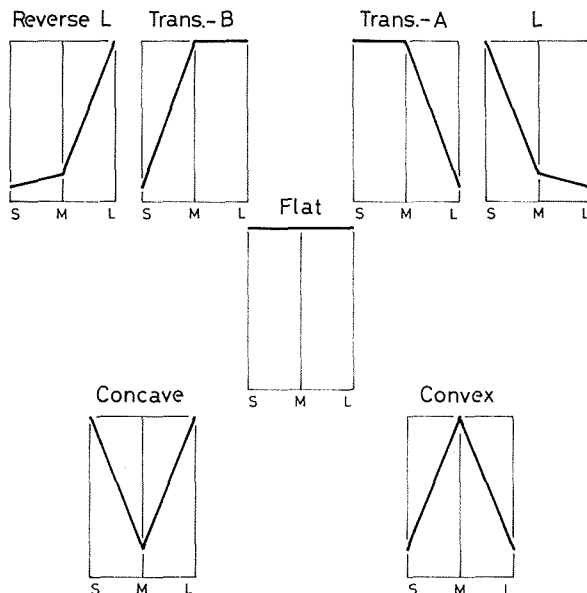

Fig. 8. Basic types of the filtering efficiency curve pattern in feeding on the three sized particles of large, middle and small. The vertical axis shows the filtering efficiency and the abscissa, the size of particles (S: small, M: middle, L: large).

rate in the present study.   A value of the filtering efficiency for one fraction is independent of the values for other fractions.   So, the filtering efficiencies of a fraction obtained under various food conditions can be compared with each other in relation to those conditions.

In filter-feeding, selection for a particular fraction should be resulted from the rejection or the pass-out of other fractions filtered at a time.   If the rejection or the pass-out does not occur, the filtering efficiency should be same for any fraction.   The difference in the filtering efficiency among the fractions represents the differences in the degree of the intensity of the rejection or the pass-out.

As a selectivity index in filter-feeding, thus, the filtering efficiency is better and more advantageous than the IVLEV's index.   On the theoretical base, the filtering efficiency is identical with the selectivity coefficient (VANDERPLOEG & SCAVIA 1979) and the retention efficiency (NIVAL & NIVAL 1976; RUNGE 1980), the availability of which has been defined theoretically (VANDER-PLOEG & SCAVIA 1979).

The pattern of the f curve is also useful for representing visually the differences and changes in size-selective feeding.   Assuming three food particles-sizes (small, middle, large), the pattern can be classified into the following seven types in relation to possible feeding behaviours (Fig. 8);

(1)   Flat type: Non-selective feeding, when the efficiency is equal between all sizes of particles.
(2)   L-shape type: Selection for small particle, by rejection against the larger particles.
(3)   Convex type: Selection for middle particle, by both of rejection against large particle and pass-out of small one, or by raptorial seizing on the middle particle alone.
(4)   Concave type: Selection for small and large particles, and inhibition for middle one, by rejection against the middle one alone and probably simultaneously by raptorial seizing on the large one, as well.
(5)   Reverse L-shape type: Selection for large particle, by pass-out of small and middle particles, or by raptorial seizing on the large one.
(6)   Transitional type-A from the flat type to the L-shape or convex type: Selection for small and middle particles, by rejection against large particle.
(7)   Transitional type-B from the flat type to the reverse L-shape or convex type: Selection for middle and large particles, by pass-out of small particle alone, or by raptorial seizing on the middle and large ones.

All of the above-described types, without the concave one, can be interpreted only by the mechanical filtering with the aid of rejection and pass-out of particles.   However, the concave type cannot be well understood without a help of raptorial seizing, because it would be impossible that the middle particle alone could be rejected in the mechanical sieving.   This type is not expected from *Daphnia*, since there has been no report that *D. longispina* can seize raptorially large food.

Raptorial feeding behaviour of calanoid copepods has been known (GAULD 1964; JØRGENSEN 1966; ALCARAZ *et al.* 1980; DONAGHAY 1980; RICHMAN *et al.* 1980).   *Eodiaptomus* seems to feed raptorially large food particles, judging from the fact that an apparent change from the small particle selection to the large particle one in December was due to an abrupt increase in the filtering and feeding rates on the second largest fraction.   Both of the transitional B-like type in December and the clear convex type in February also appear to have been caused by the raptorial seizing of large particles.   Accordingly, all of the above-mentioned seven types would

be detectable in *Eodiaptomus*, while the six types in *Daphnia*.

The filtering efficiency and its curve pattern are valid indicators for analyzing possible relationships between the food selection and food condition in connection with the feeding behaviours.

3–5. Some characteristics of the selective feeding by *D. longispina* and *E. japonicus* in Lake Biwa.

Daphnia longispina showed two different feeding patterns of food selection; one is the selection for the smallest fraction, and the other, the non-selection regarding to fraction size. It indicates that the particle-size selection is changeable in dependence on food condition. The former feeding pattern agrees with the results in many studies on selective feeding of *Daphnia* species (GLIWICZ 1969, 1977; BERMAN & RICHMAN 1974; GOPHEN et al. 1974; NADIN-HURLEY & DUNCAN 1976; INFANTE 1978). *D. longispina* in a Polish lake catches the artificial beads below 20 μm in diameter more efficiently than the larger ones (GLIWICZ 1977). The selection for the smallest fraction in the present study is a general feature of feeding by this daphnid.

Such small particles selection results from large particles rejection. The rejection efficiency can be shown by the reversal of filtering efficiency. The low filtering efficiencies, or the high rejection ones, for the largest and second largest fractions are found not only in August and September when large green algae such as *S. dorsidentiferum* and *P. Biwae* predominated exclusively in the algal compositions, but also in November when *M. solida* was the dominant alga and *S. dorsidentiferum* was the secondary dominant. In December when *M. solida* kept the dominance but *S. dorsidentiferum* was much scarce, the rejection against the two larger fractions appeared to be weakened. *Staurastrum* species are known as poorly ingested items for a daphnid because of its large size and complex shape (INFANTE 1973; LAMPERT 1977; PORTER 1977). The high density of this alga may obstruct the smooth feeding of *Daphnia* and induce the intensive rejection. On the contrary, *M. solida* seems to be primarily an edible alga. Supposedly, *D. longispina* in Lake Biwa may not be a specialist who selects always for small particles alone.

E. japonicus exhibited an apparent change in size-selection from small particles to large ones (cf. Figs. 6 & 7). It is consistent with the alteration of dominant alga from *S. dorsidentiferum* to *M. solida*. In November when a transitional feature of the change in size-selective feeding was observed, the filtering efficiency for the largest fraction (0.26 on the average) was much lower than that for the second largest one (0.84). In the two fractions, the dominant alga was same, *M. solida*, but the secondary dominant one was different; *S. dorsidentiferum* in the largest fraction, while *Sphaerocystis shroeteri* in the second largest fraction. The density of *Staurastrum* (incl. *S. tohopekarigense*) was 24.0 cells·ml^{-1} in the former fraction, but 0.4 cells·ml^{-1} in the latter. In December when *M. solida* dominated but *Staurastrum* spp. were scarce, 5.6 and 3.8 cells·ml^{-1} in the largest and second largest fractions, respectively, the filtering efficiencies were high for both fractions. *S. dorsidentiferum* is conceived to be unfavourable even for *Eodiaptomus*. To the contrary, the clear positive IVLEV's indices and the high filtering efficiencies for the largest and second largest fractions in December indicate that *M. solida* is a preferable alga. This agrees with that *Eudiaptomus sicilis* handled efficiently chainformed diatoms (BOWERS 1980; VANDERPLOEG 1981).

This suggests that the food-size selection of *Eodiaptomus* varies with the changes in the algal

species composition especially in the larger fractions of a natural phytoplankton assemblage. This selection seems to be dependent on the preference to species specific qualities of algae such as shape or palatability. The abilities of both rejecting and raptorial seizing appear to be considerably developed in *Eodiaptomus*, though how and what kinds of food qualities induce such behavioural feeding responses are unknown.

3-6. An appraisal of the fractionizing method for a feeding experiment.

The method, in which a natural phytoplankton assemblage is fractionized and the fractions are differentially labelled with radioisotope, has not been applied to the measurement of feeding rates of zooplankters on the whole phytoplankton assemblage since BOGDAN and McNAUGHT (1975). It seems to be due to not only the intricacy of the proceeding of experiment but also a question to the validity of the fractionation of phytoplankton.

A part of the latter question has been already discussed in the section 3-1. Various algal species were always observed within every fraction except the smallest one. Serious questions are whether each of the algal particles in a given fraction could be labelled at the same level of radioactivity per unit particle biomass and whether the zooplankters might feed selectively special particles alone in a fraction. These two problems cannot be solved without the aid of a way in which feeding rate on each algal species in a phytoplankton assemblage can be measured. However, such a way has not been contrived. Accordingly, the feeding and filtering rates obtained in the present experiments are overall average values for the particles in a fraction. The fractionizing method cannot provide so distinct and detailed characteristics of particle size-selection by zooplankters as the Coulter counter method. However, even the Coulter counter method has two serious weak points; food condition changes quantitatively and qualitatively during the feeding experiment because of the necessity of a long feeding period, and production of feces due to defecation and small particles due to mastication of large food particles by zooplankton conceals an actual decrease in number of the same sized food particles as such newly produced feces and particles.

The feeding and filtering rates of zooplankton on natural phytoplankton are influenced not only by algal size but also by shape, taste and other qualitative factors of food. The Coulter counter method cannot give an information on such qualitative characteristics of algal particles except size. From an algal fraction, to the contrary, some qualitative factors such as shape, species and perhaps mean chemical composition of food particles can be determined. These informations would be very useful for analyzing and understanding the selective-feeding behaviours of zooplankton in nature.

Advantageously, feeding and filtering rates of each zooplankton species in a plankton community can be estimated in relation to the zooplankton size. Photosynthetic activity of fractionized phytoplankton can be measured (NAKANISHI 1976). Thus, the fractionizing method enables to compare between zooplankton grazing on and primary production of a certain size-fraction in a natural phytoplankton assemblage. Such comparative study will reveal out in more detail not only the structure and function of energy and matter flows from phytoplankton to herbivorous zooplankton in a lake ecosystem, but also the grazing effect on phytoplankton succession in nature.

4. Acknowledgements

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