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Feeding kinetics of *Brachionus plicatilis* fed *Isochrysis galbana*

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Abstract

Clearance and ingestion rates of *Brachionus plicatilis* were measured using ^{14}C-labeled *Isochrysis galbana* Tahiti. Experiments were conducted at 20–22 °C, 20 ppt salinity, and algal concentrations ranging from 0.13–64 mg C l^{-1}. Clearance rates were constant and maximal at concentrations <2 mg C l^{-1}, with maximum rates ranging from 3.4–6.9 μl ind.$^{-1}$ hr^{-1}. The ingestion rate varied with food concentration, and was described by a rectilinear model. The maximum ingestion rate varied considerably, and was dependent on the growth rate of the rotifers. Depending on the pre-conditions, *B. plicatilis* ingested about 0.5 to 2 times its body carbon per day at saturating food concentrations.

Introduction

A wealth of information has been published on feeding in the rotifer genus *Brachionus*, primarily in *B. calyciflorus* (see Starkweather, 1980). Although many feeding studies have also been done on *B. plicatilis*, most have employed methodology requiring several hours duration which can result in spurious results (cf. Dewey, 1976; Yufera & Pascual, 1985; Yamasaki & Hirata, 1986). Doohan (1973) applied the more sensitive radiotracer technique to measure clearance and ingestion rates in this species. Her results, however, were quite variable and the ingestion rates were probably underestimated because of the long incubation time used. Thus, knowledge of the feeding kinetics of *B. plicatilis* is incomplete. Because *B. plicatilis* is cultured as start food for marine fish larvae (Howell, 1973; Gatesoupe, 1987; Robin, 1987; Theilacker, 1987), this information would ultimately aid aquaculturists.

This paper describes a series of experiments using radio-actively labeled algae to measure clearance and ingestion rates of *B. plicatilis*. The results demonstrate the effect of food concentration and growth rate on rotifer feeding kinetics.

Materials and methods

Brachionus plicatilis was obtained from stock cultures at SINTEF Aquaculture Center in Trondheim, and maintained on a diet of *Isochrysis galbana* (Tahiti) for at least one week before experiments commenced. Stock rotifer cultures were continuously aerated and kept at 20 °C and 20 ppt salinity. The average length of adults of this strain is about 150 μm. *Isochrysis* cultures were grown in semi-continuous culture diluted at 0.33 day^{-1} using *f* media (Guillard, 1975). Radioactively labeled algae were prepared as follows: 20 μCi $NaH^{14}CO_3$, unlabeled $NaHCO_3$ corresponding to 50 mg C l^{-1}, and about 25 ml new *f* media were added to approximately 25 ml *Iso-*

chrysis from the stock culture (ca. 120 mg C l^{-1} before dilution) in a 125 ml glass-stoppered bottle. The bottle was placed in constant light at about 60 μEin m^{-2} sec^{-1}.

B. plicatilis used for the feeding experiments was taken from the stock culture, placed in a 50 μm sieve in a beaker, and concentrated by carefully pipeting most of the excess water out of the beaker. The rotifers were pre-acclimated with non-radio-active *Isochrysis* at the desired concentration for 1–2 hours. All experiments were conducted at 20–22 °C, 20 ppt salinity, and about 15 μEin m^{-2} sec^{-1} light intensity. Sea water used for dilution and rinsing had been previously filtered through a Whatman GF/C filter, autoclaved, cooled to experimental temperature, and aerated.

After the pre-feeding incubation, the rotifers were concentrated as before and added to radioactive algae in flasks. Volume and incubation time depended upon the type of experiment and the flasks were agitated periodically during incubation (Schlosser & Anger, 1982). At the end of the experiment, replicate 3–5 ml samples were taken and filtered by gravity through 50 μm Nitex netting in 2.5 cm diameter Millipore filtering units, and rinsed with 50 ml 20 ppt sea water. The rotifers were maintained in about 3 ml of water throughout the rinsing. The Nitex netting was then placed in a scintillation vial. It took about 2 min from taking the sample from the flask to placing the Nitex netting in the scintillation vial.

Replicate samples were taken for determination of specific activity (3 × 100 μl) and for rotifer counts (5 × 200 μl). One hundred μl H_2O_2 was added to each vial to remove color. After 2 hr, 20 μl 0.5 N HCl was added to the specific activity samples to remove inorganic ^{14}C and 5 ml scintillation cocktail (Optifluor, Packard) was thereafter added to each vial. Samples were counted with a Nuclear Chicago Isocap 300 liquid scintillation counter for 10 min using the external standard ratio. Control experiments revealed similar counts and counting efficiencies of rotifer samples treated as above with the inclusion of adding tissue solubilizer (200 μl Soluene-350, Packard). To estimate background radioactivity on the screens we used the radioactive algal feeding suspensions without added animals and processed samples as above. These controls were not significantly different from zero time samples with animals. Heat-killing of the rotifers before filtration yielded irreproducible results because the algae stuck to rotifers and netting.

Three different types of experiments were conducted to describe the feeding kinetics of *B. plicatilis*. A time course experiment was conducted at two food concentrations (5 and 15 mg C l^{-1}) to determine the gut passage time of the rotifer. Twenty measurements between 0 and 60 min were taken. Following the results of this experiment, subsequent feeding studies were conducted for 15–20 min. Two functional response experiments were done to determine the clearance and ingestion rates of the rotifers at different food concentrations. Rotifer stock cultures used in these experiments were fed daily in the first experiment and every other day in the second experiment. Finally, maximum ingestion rates were determined at 30 mg C l^{-1} throughout a batch culture experiment to observe variability in the ingestion rates following different long term preacclimation times. To obtain rotifers with close to zero growth rate, they were starved for 3 days prior to starting this experiment.

Results

The results of the time series experiment (Fig. 1) revealed that the gut passage time of *B. plicatilis* was dependent on food concentration. As indicated by the abrupt change in the slope of the regression lines, the gut passage time was approximately 30 and 45 min at 5 and 35 mg C l^{-1}, respectively. We therefore chose a feeding interval of 15–20 min for the other experiments.

The clearance rates for both functional response experiments (Fig. 2) were highest and constant at food concentrations below 1–2 mg C l^{-1} and decreased with increasing food concentration. Ingestion rates for both functional response experiments increased linearly with increasing food concentration up to a maximum and thereafter remained constant at higher food

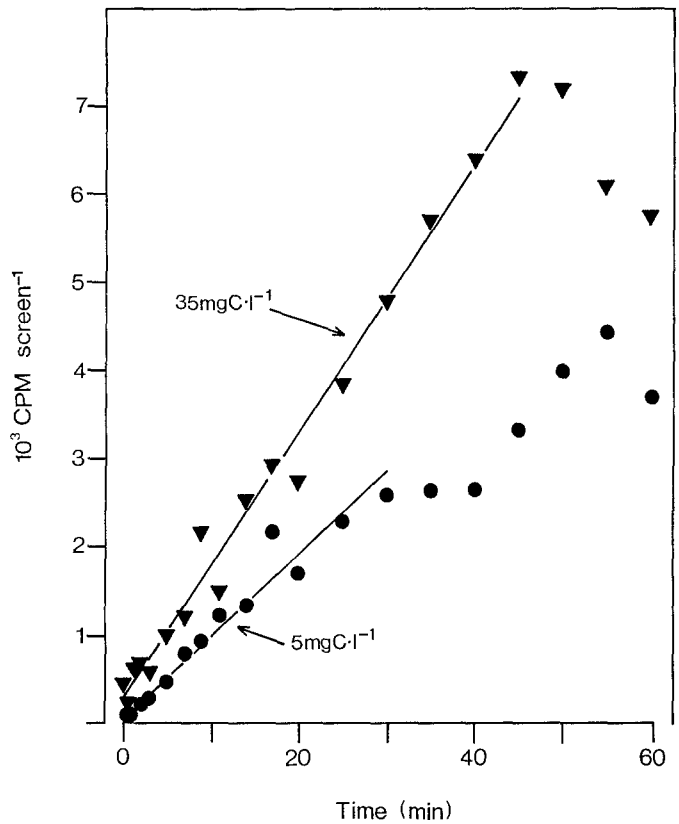

Fig. 1. Accumulation of radioactive food (^{14}C *I. galbana*) by *B. plicatilis* over time at two food concentrations. Gross CPM per Nitex screen is given on the ordinate.

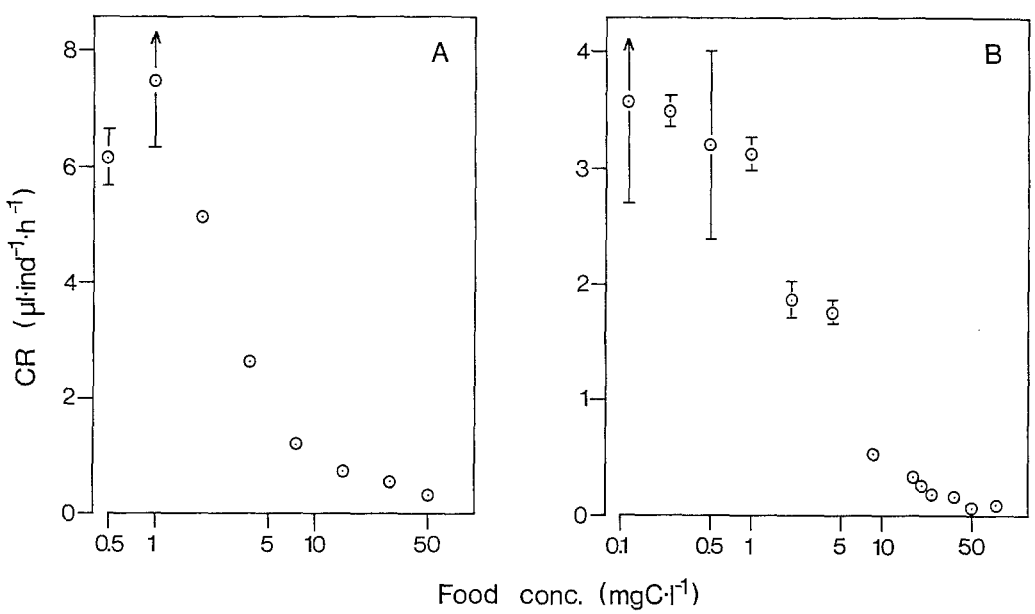

Fig. 2. Clearance rates (CR) of *B. plicatilis* for functional response experiment 1 (A) and 2 (B). Standard errors indicated when larger than symbol.

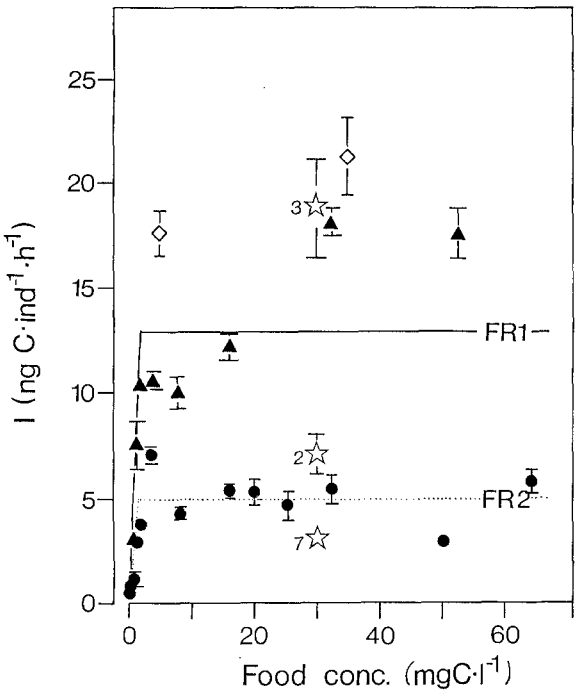

Fig. 3. Ingestion rates of *B. plicatilis* as a function of concentrations of *I. galbana*: Time Series experiment (calculated from the slope of the curve before the inflection points shown in Fig. 1) (◇), both Functional Response experiments (FR 1 ▲ and FR 2 ●), and the Batch experiment (☆). Day number indicated for the batch experiment. Error bars indicate ± SE. Lines represent model curves for the two functional response experiments (cf. Table 1).

concentrations (Fig. 3). Ingestion and clearance rates were higher in the first experiment than in the second experiment at all food concentrations. The ingestion data could be described by rectilinear, Ivlev and Michaelis-Menten models ($P < 0.001$). In Table 1 results from the rectilinear regressions are presented. We have chosen this model in the further treatment because of its simplicity (zero order approximation), and the biologically relevant parameters in the model – the maximum clearance rate (CR_{max}) and maximum ingestion rate (I_{max}). A 2.6 and a 2.0 times difference was recorded in I_{max} and CR_{max}, respectively, between the two experiments and the differences were significant (Table 1). The incipient limiting concentration (ILC) was, on the other hand, not significantly different in the two experiments.

In the batch experiment the rotifers were growing exponentially from day 1 to day 4 when the food was depleted, and the culture reached stationary phase at day 5–6 (Fig. 4). The maximum ingestion rate (I_{max}) was intermediate on day 2, increased by a factor of 2.7 on day 3, and decreased dramatically upon entering stationary phase (Fig. 3). Overall, a 6 fold difference was recorded in I_{max} during this experiment. No sig-

Table 1. Results from fitting a rectilinear model to the data of ingestion rate (I) as a function of food concentration (C) in the two functional response experiments. Maximum ingestion rate (I_{max}) and maximum clearance rate (CR_{max}) was fitted by zero-order approximation, and incipient limiting concentration (ILC) was calculated as I_{max}/CR_{max}. Parameters given with one standard error. Differences between parameters were evaluated using the Student's t-test. For comparison Dewey's (1976) data for *B. plicatilis* fed *I. galbana*, are included.

$I = CR_{max} \cdot C$ $\quad (C \leq ILC)$
$I = I_{max}$ $\quad (C \geq ILC)$

Exp.	CR_{max} (μl ind.$^{-1}$ hr^{-1})	I_{max} (ng C ind.$^{-1}$ hr^{-1})	ILC (mg C l^{-1})	P
1	6.81 ± 0.68 (n = 12)	12.93 ± 0.63 (n = 36)	1.90 ± 0.21 (n = 46)	<0.001
2	3.38 ± 0.31 (n = 18)	4.93 ± 0.22 (n = 54)	1.46 ± 0.15 (n = 70)	<0.001
	$P < 0.001$	$P < 0.001$	$P > 0.05$	
Dewey	6.01 ± 0.38 (n = 10)	19.1 ± 1.9 (n = 6)	3.18 ± 0.37 (n = 16)	

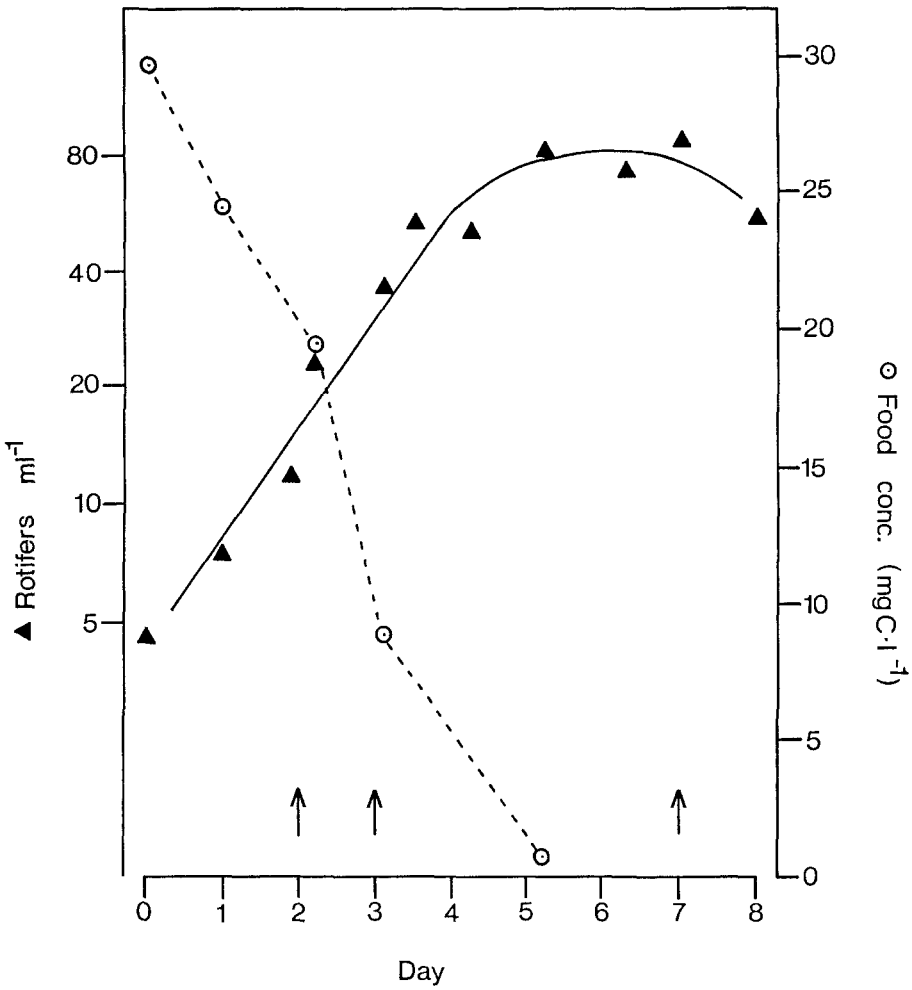

Fig. 4. Number of rotifers and *I. galbana* concentration versus day for the batch experiment. Arrows indicate days on which ingestion rates were measured.

nificant differences were recorded in the length of the animals, that averaged 155 ± 4 μm (± SD).

Discussion

The gut passage time is known to vary with species, temperature, food concentrations, and the nutritional state of the animals (Starkweather & Gilbert, 1977b). It probably also varies for foods with different assimilation efficiencies. Reported gut passage times in other species of rotifers under varying conditions ranges from 2–20 min (cf. Resvoi, 1926; Starkweather & Gilbert, 1977b). Our results with *B. plicatilis* are in agreement with the higher values of this range.

It has been suggested that filter feeders would benefit by reducing their filtering rate at very low food concentrations to ensure energy optimization (Mullin *et al.*, 1975; Lehman, 1976). No reduction in clearance rate was found in the present study for food concentrations down to 0.13 mg C l^{-1}. Our results are in accordance with most other studies on rotifer feeding (e.g. Erman 1956; Dewey, 1976; Starkweather & Gilbert, 1977a; Chotiyaputta & Hirayama, 1978). The maximum clearance rates reported by Dewey (1976) for *B. plicatilis* fed *Isochrysis galbana* were

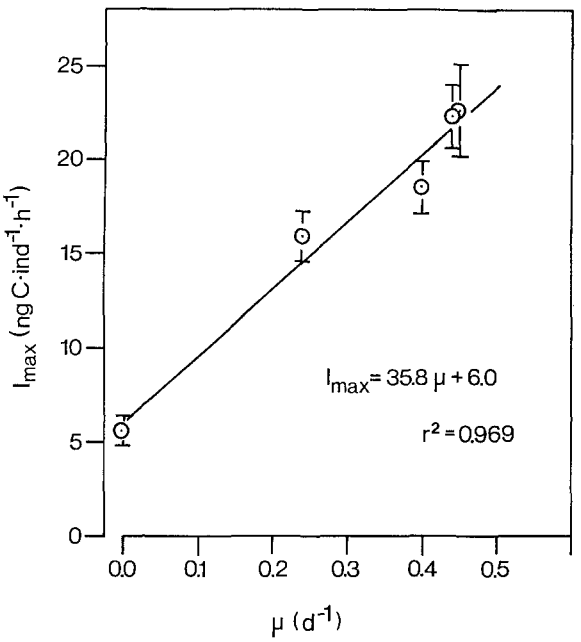

Fig. 5. Maximum ingestion rate (I_{max}) at saturating food concentrations as a function of specific growth rate (μ) for *B. plicatilis* fed *Dunaliella tertiolecta*, assuming $\mu = 0$ for starved animals. Data are from Dewey (1976) and 95% CI are given for I_{max}.

intermediate to those estimated for the two functional response experiments (Table 1). The ingestion rates obtained on days 2 and 7 in the batch culture experiment were similar to I_{max} of functional response experiment 2, whereas those obtained on day 3 of the batch experiment and for the two time series experiment were comparable to that obtained in functional response experiment 1. The results presented in Fig. 3 indicate that individual *B. plicatilis* can ingest 0.5–2 times their body carbon per day at saturating food concentrations (assuming 0.23 μg C rotifer^{-1}).

The results obtained in the batch culture experiment suggest that the maximum ingestion rates were related to the physiological status of the rotifers. Starved rotifers exhibited 6 times lower I_{max} than well fed rotifers, although the starved rotifers were allowed to equilibrate their gut before the experiment (see methods). At least three days at saturating food concentrations are needed before the rotifers are capable of maintaining the highest I_{max}. This also agrees with the 2.6 times difference in I_{max} which was recorded in the two functional response experiments. The rotifers used in experiment 2 were fed every second day, which caused the rotifers to experience large oscillations in the food concentration and periods of starvation. We found no significant correlations between maximum ingestion rates and size, swimming speed, egg ratio, or the abundance of rotifers in the experimental containers.

Dewey (1976) has reported similar variability in I_{max} and attributed it to size differences in the rotifers. Her results with *B. plicatilis* fed *Dunaliella tertiolecta* can be re-interpreted, and in Fig. 5 we have plotted I_{max} as a function of the specific growth rate. There is a significant linear relation between the two parameters ($P < 0.005$), which supports our hypothesis that the feeding kinetics of *B. plicatilis* is affected by the physiological status of the animals. We therefore conclude that the feeding rate of *B. plicatilis* is not only a function of the food concentration, but also of the growth rate of the rotifer. Further research into the feeding biology of *B. plicatilis* and other rotifers should take the long term (days) pre-feeding history of the animals into account.

Acknowledgements

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