The significance of some methodological effects on filtration and ingestion rates of the rotifer *Brachionus plicatilis**

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ABSTRACT: Filtration rate (F) and ingestion rate (I) were measured in the rotifer *Brachionus plicatilis* feeding on the flagellate *Dunaliella* spec. and on yeast cells (*Saccharomyces cerevisiae*). 60-min experiments in rotating bottles served as a standard for testing methodological effects on levels of F and I . A lack of rotation reduced F values by 40 %, and a rise in temperature from 18° to 23.5°C increased them by 42 %. Ingestion rates increased significantly up to a particle (yeast) concentration of ca. 600–800 cells · μl^{-1} ; then they remained constant, whereas filtration rates decreased beyond this threshold. Rotifer density (up to 1000 ind · ml^{-1}) and previous starvation (up to 40 h) did not significantly influence food uptake rates. The duration of the experiment proved to have the most significant effect on F and I values: in 240-min experiments, these values were on the average more than 90 % lower than in 15-min experiments. From this finding it is concluded that ingestion rates obtained from short-term experiments (60 min or less) cannot be used in energy budgets, because they severely overestimate the actual long-term feeding capacity of the rotifers. At the lower end of the particle size spectrum (2 to 3 μm) there are not only food cells, but apparently also contaminating faecal particles. Their number increased with increasing duration of experiments and lead to an underestimation of F and I . Elemental analyses of rotifers and their food suggest that *B. plicatilis* can ingest up to 0.6 mJ or ca. 14 % of its own body carbon within 15 min. The long term average was estimated as 3.4 mJ · ind⁻¹ · d⁻¹ or ca. 75 % of body carbon · d⁻¹.

INTRODUCTION

Standardized techniques are needed to measure the filtration and ingestion rates in planktonic animals. Such information is required for a research project of the Biologische Anstalt Helgoland, aiming to experimentally investigate pelagic food web dynamics in the marine environment. The present study attempts to analyze some methodological aspects influencing such measurements. The rotifer *Brachionus plicatilis* was used as a test organism because it is available in mass cultures. In addition, knowledge of its nutrition is of interest for that part of the research project concerned with the development of cultivation techniques.

Aquaculturists have paid particular attention to large scale production of this

* Contribution to research project "Experimentelle Marine Ökosystemanalyse" sponsored by the Bundesministerium für Forschung und Technologie, Bonn (Grant No. MFU – 0328/1)

species (for recent review of this aspect see Theilacker & McMaster, 1971; Furukawa & Hidaka, 1973; Yúfera & Pascual, 1980; Gatesoupe & Luquet, 1981; Gatesoupe & Robin, 1981). Its filtration rate was studied by Hirayama & Ogawa (1972), Doohan (1973), Dewey (1976), Chotiyaputta & Hirayama (1978) and Capuzzo (1979). Erman (1956, 1962), Gal-kovskaja (1963), Halbach & Halbach-Keup (1974), Gilbert & Starkweather (1977), Starkweather & Gilbert (1977), and Starkweather & Frost (1979) made similar investigations on other *Brachionus* species.

MATERIAL AND METHODS

Both the flagellate *Dunaliella* spec. (isolated from Helgoland plankton) and the rotifer *Brachionus plicatilis* were cultivated separately in 10-l flasks with filtered, sterilized sea water (Millipore: $0.45\text{ }\mu\text{m}$; ca. 32‰; 23.5°C) applying slight aeration. Rotifers were fed twice daily with ca. 2.5 g bakery yeast (*Saccharomyces cerevisiae*); water was changed once a week. The algae were grown in F/2 medium (Guillard & Ryther, 1962). A photoperiod of LD 16:8 h was used throughout.

Cultures were allowed to adapt to the experimental temperature(s) for at least three days before they were used in experiments. Since the physiological stage of the algae (exponential vs. stationary phase) exerts an influence on the filtration rate of the rotifers (Chotiyaputta & Hirayama, 1978), only algal cultures which had reached concentrations of 1,000 to 2,000 cells $\cdot\text{ }\mu\text{l}^{-1}$ were used. 24 h before onset of the experiment they were diluted with sterile, Millipore-filtered ($0.22\text{ }\mu\text{m}$) sea water to the approximate concentrations desired. These suspensions were stored in the dark at the experimental temperature(s). Their actual concentrations were determined immediately before the experiment was started. A Coulter Counter model TAPII (calibration: 330.3; tube aperture: $100\text{ }\mu\text{m}$) was employed. The mean value of 12 readings was used as initial concentration (c_0).

If *S. cerevisiae* was to be used as a test food, 0.1 to 0.4 g bakery yeast were added to 1 l seawater. This mixture (ca. 500 to 3,000 cells $\cdot\text{ }\mu\text{l}^{-1}$) was kept in suspension for ca 12 h on a magnetic stirrer ($600\text{ r} \cdot \text{min}^{-1}$; experimental temperature; with aeration). The density of yeast cells was measured in the same way as in *Dunaliella* spec. Microscopic examination revealed that after 4 h only very few cell aggregates (2–3 cells) were still present; no changes of the suspension were observed within the next 24 h.

One hour before the experiment began, the rotifer culture was poured through two successive screens (150 and $90\text{ }\mu\text{m}$ mesh size). The first one retained large individuals and wastes, the second one the experimental animals. By means of a squeeze bottle with Millipore-filtered sea water they were washed and then flushed into an erlenmeyer flask where they remained, slightly aerated, at experimental temperature until the experiment was started. Very small individuals, eggs, and food particles passed both sieves and were discarded. In this way it was achieved that in all experiments rotifers of similar size were used.

Densities of *B. plicatilis* cultures could be measured with a Coulter Counter (calibration: 171.8; tube aperture: $560\text{ }\mu\text{m}$). In preliminary experiments it was observed that a certain amount (up to 50 %) of the rotifers actively attached themselves to the glass walls of the beaker and passively adhered to the water surface. This systematic error could be substantially reduced in the following way: a sample (15 ml) of the stirred and aerated culture was taken by means of a calibrated pipette, the rotifers were retained on a $45\text{ }\mu\text{m}$

screen, and flushed into a 100 ml beaker with 50 ml Millipore-filtered seawater. A few drops of formaldehyde (40 %) and of a detergent were added, and the sample was stirred. In this way, the rotifers were homogeneously distributed and the counting error became relatively small (less than 10 % standard deviation as revealed by five measurements). For each determination of rotifer density at least 5 samples were counted. Values having ≥ 10 % standard deviation were disregarded.

The experiment was started as follows: 15 ml rotifer culture were pipetted onto a 45 μm screen (as above), and flushed from there into the experimental flask (capacity: 100 ml) with at least 50 ml food suspension (algae or yeast), which had been prepared and measured as described above. With the adjusted amount of food suspension, a density of ca. 50 rotifers $\cdot \text{ml}^{-1}$ was achieved in all standard experiments. These always comprised 8 flasks with rotifers plus food (experimental replicates) and 4 equally treated control replicates without animals. The flasks were closed with screw caps and mounted in an incubator, where they rotated in the dark with $10 \text{ r} \cdot \text{min}^{-1}$ over their longitudinal axis. After 60 min the rotifers were screened out on 45 μm gauze, and the concentrations of food particles were counted again in all 12 flasks. Temperature was usually $23.5 \pm 0.6^\circ\text{C}$, and $18 \pm 0.2^\circ\text{C}$ as an exception (only one experiment with *Dunaliella* spec. as food).

Filtration and ingestion rates of *B. plicatilis* were measured using this standard technique, and compared with those under modified conditions, which will be briefly described below (chapter "Results"). Filtration rate is defined as volume swept clear per unit of time. For the calculation of average filtration (F ; $\mu\text{l} \cdot \text{ind}^{-1} \cdot \text{min}^{-1}$) and ingestion rates (I ; $\text{cells} \cdot \text{ind}^{-1} \cdot \text{min}^{-1}$) the following equations were used (Gauld, 1951; Hargis, 1977):

$$F = \frac{v}{n} \left(\frac{\ln c_o - \ln c_t}{t} - A \right)$$

$$A = \frac{\ln c_o - \ln c'_t}{t}$$

$$I = F \cdot \sqrt{c_o \cdot c_t}$$

where c_o and c_t are initial and final food concentrations ($\text{cells} \cdot \mu\text{l}^{-1}$), t is time (duration of the experiment in min), and n is the number of rotifers (ind) in volume v (μl). A is a correction for changes in the control with final concentration c'_t after time t . The expression $\sqrt{c_o \cdot c_t}$ represents the geometric mean of food concentration during time t .

The biomass of *B. plicatilis* and *S. cerevisiae* was determined in samples with known volumes and concentrations, collected on pre-weighed glass fiber filters (Whatman: GF/C). The material was briefly rinsed with distilled water and freeze-dried (at least 3 h at 10^{-2} m bar) in a GT2 (Leybold-Heraeus) apparatus. Dry weight was measured on an Autobalance AD-2 (Perkin-Elmer) to the nearest 0.1 μg . Carbon (C) and nitrogen (N) contents were determined in an Elemental Analyzer model 1106 (Carlo Erba Science) using Cyclohexane-2,4-dinitrophenyle-hydrazone as a standard. Care was taken that the sample dry weight was between 0.2 and 2 mg, which is the range of maximum accuracy in the CHN analyzer.

The data on *Dunaliella* spec. were obtained from Dr. E. Hagmeier (pers. comm.), who used identical techniques and equipment in his analyses.

RESULTS

Experiments with *Dunaliella* spec.

Information on the influence of flask rotation during the experiment was desired to allow comparison with literature data obtained from experiments without agitation. Identical cultures of *Brachionus plicatilis* and *Dunaliella* spec., respectively, were used in parallel experiments with different food concentrations (ca. 50 to 200 cells $\cdot \mu\text{l}^{-1}$) but similar rotifer densities (44 ± 4 to 52 ± 5 ind $\cdot \text{ml}^{-1}$), with and without rotation. The latter part of the experiment was carried out in 100 ml beakers; as in the standard procedure, 8 experimental and 4 control replicates were run to calculate one average value for filtration rate. The temperature in all these experiments was $18^\circ \pm 0.2^\circ\text{C}$.

Fig. 1. *Brachionus plicatilis*: Filtration rate (left graph) and ingestion rate (right graph) in relation to food concentration (*Dunaliella* spec.), with and without rotation of experimental flasks

Figure 1 shows that filtration rate was independent of algal concentration in the range observed (regression and correlation coefficients, m and r , equal zero), and consequently ingestion rates increased with increasing food density. This pattern was found under both experimental conditions; however, the absolute amounts of F and I were strongly influenced by absence or presence of rotation: lack of water movement reduced the average F values by 40 %.

Parallel to these experiments others were carried out at 23.5°C under standard conditions (with rotation). They suggest a pattern identical to that observed at 18°C : F values did not significantly vary with food concentration, whereas I values increased as a linear function of algal density (Fig. 2). The slopes of the regression lines for I at 18° and 23.5°C were not significantly different from each other. The average filtration rate measured at the higher temperature was $0.122 \pm 0.013 \mu\text{l} \cdot \text{ind}^{-1} \cdot \text{min}^{-1}$, i.e. 42 % more than at 18°C .

Fig. 2. *Brachionus plicatilis*: Ingestion rate in relation to food concentration (*Dunaliella spec.*), at 18° and 23.5°C

Fig. 3. *Brachionus plicatilis*: Filtration rate (left graph) and ingestion rate (right graph) in relation to food concentration (*Saccharomyces cerevisiae*)

Experiments with *Saccharomyces cerevisiae*

All experiments with yeast cells as food for *B. plicatilis* were carried out at 23.5°C. In the experimental series testing the effect of different food concentrations, the variation of results was very high (Fig. 3). There was no obvious relationship between filtration rate and particle concentration below ca. 600 to 800 cells · μl^{-1} . The average F value in this range was $0.063 \pm 0.012 \mu\text{l} \cdot \text{ind}^{-1}$; at densities above ca. 800 cells · μl^{-1} , F figures dropped. This pattern resulted in a linear increase of ingestion rate up to a concentration of ca. 800 cells · μl^{-1} (Fig. 3, right graph). At higher particle densities ingestion rate tended to be constant (average: ca. 41 cells · $\text{ind}^{-1} \cdot \text{min}^{-1}$).

Another test was made to investigate possible effects of starvation. In 5 experimental sets (again with 8 replicates and 4 controls in each) rotifers which had been starved for 10, 20, 23, 27, and 40 h were tested at food densities of 242 to 250 cells · μl^{-1} . Filtration rates of 0.094 ± 0.005 to $0.109 \pm 0.005 \mu\text{l} \cdot \text{ind}^{-1} \cdot \text{min}^{-1}$ and ingestion rates of 19.5 ± 0.8 to 22.2 ± 2.8 cells · $\text{ind}^{-1} \cdot \text{min}^{-1}$ were found. There was no relationship (correlation and regression coefficients close to zero) between the duration of starvation and feeding rates measured in these 60-min experiments.

The following experimental series was designed to evaluate the effect of experiment duration: in 5 experiments lasting 15 to 240 min strongly decreasing feeding rates were observed with increasing duration (Fig. 4, regression curves and equations No. I). Average values obtained from 240-min experiments were more than 90 % lower than those found in 15-min experiments.

Fig. 4. *Brachionus plicatilis*: Filtration rate (left graph) and ingestion rate (right graph) in relation to experiment duration, including (I) and excluding (II) data from Coulter Counter channel 5

Analysis of particle size-frequency distribution revealed that the absolute number of particles detected after the experiment in Coulter Counter channel 5 increased considerably with increasing experimental time. Above 60 min it was even higher than before the experiment (up to $150 \text{ cells} \cdot \mu\text{l}^{-1}$). This pattern was not found in controls without animals. When figures recorded in channel 5 were disregarded, calculation of feeding rates consequently yielded far higher values (Fig. 4, regressions No. II). The meaning of this observation will be discussed below.

Another test was made to evaluate effects of rotifer density on average filtration rates. Five experiments were carried out at different times (with different rotifer populations), always applying standard techniques: 50 to 70 *B. plicatilis* $\cdot \text{ml}^{-1}$, 540 to 650 yeast cells $\cdot \mu\text{l}^{-1}$, duration 60 min, 23.5°C , rotation, 8 replicates plus 4 controls. Parallel to these were 6 other experiments which only differed in rotifer density: 150, 300, 400, 650, and 1,000 ind $\cdot \text{ml}^{-1}$. Mean filtration rate in all 10 experiments was $0.066 \pm 0.015 \mu\text{l} \cdot \text{ind}^{-1} \cdot \text{min}^{-1}$. Differences observed within pairs of experiments (with different rotifer density) were smaller than differences in succeeding standard experiments. This was confirmed by 2-way analysis of variance: rotifer concentration had no significant effect on individual filtration rates ($F = 0.04$; $p = 0.85$), whereas differences between experiments carried out on different days were highly significant ($F = 14.3$; $p = 0.01$).

Table 1 shows the results of measurements of dry weight (DW), carbon (C), and nitrogen (N). Energy values were computed from relative and absolute carbon using the conversion equation given by Salonen et al. (1976).

Table 1. Biomass in dry weight (DW), carbon (C), nitrogen (N), and energy (Joules) of rotifers (*B. plicatilis*), yeast (*S. cerevisiae*), and flagellates (*D. spec.*)

	<i>Brachionus plicatilis</i>	<i>Saccharomyces cerevisiae</i>	<i>Dunaliella spec.</i>
Dry weight (ng $\cdot \text{ind}^{-1}$)	280	$26.8 \cdot 10^{-3}$	$82.4 \cdot 10^{-3}$
C (% DW)	41.5	45.1	47.9
C (ng $\cdot \text{ind}^{-1}$)	116	$12.1 \cdot 10^{-3}$	$39.5 \cdot 10^{-3}$
N (% DW)	9.3	8.7	15.8
N (ng $\cdot \text{ind}^{-1}$)	26	$2.3 \cdot 10^{-3}$	$13.0 \cdot 10^{-3}$
Energy (mJ $\cdot \text{ind}^{-1}$)	4.3	$0.47 \cdot 10^{-3}$	$1.57 \cdot 10^{-3}$
(J $\cdot [\text{mgDW}]^{-1}$)	15.4	17.4	19.1

When maximum ingestion rates of *B. plicatilis* at 23.5°C are converted to carbon uptake, some interesting comparisons become possible: feeding on *Dunaliella spec.* at a density of $226 \text{ cells} \cdot \mu\text{l}^{-1}$, the rotifers ingested up to ca. $24 \text{ cells} \cdot \text{ind}^{-1} \cdot \text{min}^{-1}$ (Fig. 2) or 49 % of their own carbon weight per h. Such high figures were never observed during

60-min experiments with yeast as food: the maximum value found under such conditions was $46 \text{ cells} \cdot \text{ind}^{-1} \cdot \text{min}^{-1}$ (Fig. 3) or 29 % of rotifer C $\cdot \text{h}^{-1}$, due to the lower C content of yeast as opposed to the flagellates. At very high food densities in short term experiments (Fig. 4) up to ca. $90 \text{ yeast cells} \cdot \text{ind}^{-1} \cdot \text{min}^{-1}$ were consumed, i.e. 14 % of rotifer C within 15 min. Extrapolation of such figures should lead to a considerable over-estimation of feeding rates. In long term experiments ingestion rates decrease by an order of magnitude (Fig. 4). If a long term average of ca. $5 \text{ cells} \cdot \text{ind}^{-1} \cdot \text{min}^{-1}$ (see Fig. 4, curve No. I) is assumed, *B. plicatilis* should ingest ca. 3.1 % of its own carbon weight per h or 75 % per day. Converted to energy, this means an uptake of $3.4 \text{ mJ} \cdot \text{ind}^{-1} \cdot \text{d}^{-1}$.

DISCUSSION

Most literature data on feeding rates of *B. plicatilis* were evaluated to answer questions other than in the present paper: Chotiyaputta & Hirayama (1978) were mainly interested in quantifying particle selection, which had been described in principle by Gilbert & Starkweather (1977). Doohan (1973) and Dewey (1976) studied food uptake as a part of energy budget. Theilacker & McMaster (1971), Hirayama & Ogawa (1972), and Furukawa & Hidaka (1973) calculated ingestion rates from food consumption in mass cultures in order to define conditions of cultivation. Capuzzo (1979) investigated pollution effects on feeding and other life processes. All these authors used more or less different methods, and many possible effects of methodological details may have influenced their results and thus the comparability of their data. One such effect was reported by Chotiyaputta & Hirayama (1978): senescent food algae (*Chlamydomonas* spec.) reduce filtration rates in *B. plicatilis*. Therefore, we checked the condition of our algal cultures before using them in experiments.

Even the method of calculation can be a problem: Chotiyaputta & Hirayama (1978), for example, used a formula for the calculation of ingestion rates which they mistakenly attributed to Gauld (1951), and which can lead to considerable over-estimation of food uptake. Instead of using the geometric mean of c_o and c_t they calculated an "initial concentration of phytoplankton" as the average (arithmetic mean) of actual c_o and the final concentration in the control (called c_t in our paper). If the control did not change much, Chotiyaputta & Hirayama (1978) calculated something like an initial ingestion rate; which must be much higher (at least 20 %) than the actual mean value; the amount of over-estimation depends on the difference between c_o and c_t and thus on the actual filtration rate, duration of the experiment, and related variables.

Temperature is known to influence feeding rates. This effect is strong in *B. plicatilis*: An increase from 18° to 23.5°C enhanced filtration rates (F) by 42 %. This means that data obtained at varying "room temperature" may only be used as rough estimates.

Water movement is also an important factor: lack of rotation reduced F values considerably (by 40 %). If aeration is applied for agitation, this must be controlled very carefully, because its strength might be crucial in the results. Mechanical agitation provided by a shaking apparatus, a rotating device etc. should be preferred.

Comparison of our results obtained with flagellates and with yeast suspensions confirms that feeding rates (expressed either as volume swept clear or as amount of carbon per individual per unit of time) strongly depends on both quality and quantity of

food. At low particle concentrations F values remained constantly high, and ingestion rates (I) consequently increased. At higher densities F decreased and I remained constantly high (Fig. 3). This pattern was not observed in experiments with *Dunaliella* spec. as food (Fig. 1), probably because the range of particle concentrations tested was too low (maximum concentrations $208 \text{ algal cells} \cdot \mu\text{l}^{-1}$ vs. $1776 \text{ yeast cells} \cdot \mu\text{l}^{-1}$). The threshold concentration where this change of trends in F and I takes place is influenced by the kind and condition of food (Chotiyaputta & Hirayama, 1978), and most probably by the duration of the experiment.

Starvation periods lasting up to 40 h did not influence later food uptake rates in 60-min experiments. Preliminary experiments, however, suggest that such lack of food may enhance initial F and I values measured within 15-min time spans. In longer lasting experiments there appears to be a saturation effect (cf. Fig. 4) masking the influence of previous starvation.

The density of *B. plicatilis* also proved to be of minor importance for average individual feeding rates, if a range from 50 to 1,000 rotifers $\cdot \text{ml}^{-1}$ is considered.

Size frequency analysis of food particles revealed that there is a counting problem at the lower end of the particle spectrum: channel 5 of the Coulter Counter apparently does not only record food particles in the size range 2.5 to $3.2 \mu\text{m}$ diameter, i.e. the smallest yeast cells, but probably also the largest faecal pellets produced by *B. plicatilis*. This means that all measurements including channel 5 underestimate feeding rates, whereas those disregarding it will overestimate them. The error of estimation (see differences between curves I and II in Fig. 4) strongly increases with increasing duration of the experiment.

The factor influencing feeding rates most significantly besides particle concentration and temperature, is in general the duration of the experiment (Fig. 4). The longer this time span is, the lower the average filtration and ingestion rates are, presumably due to a saturation effect. The significance of this effect presumably increases with increasing particle concentration. Therefore it should be clearly defined as to whether a short-term potential (i.e. a maximum feeding rate possible for a short period) or long-term feeding is to be described. If the goal of an investigation is to achieve an energy budget, the latter value is more of interest. Doohan (1973) used figures obtained from 60-min experiments in her energy budget for *B. plicatilis*. These values for ingestion rate compare favourably with ours, when also 60-min experiments are taken for comparison, but they are 10 times higher than our estimate for the long-term energy demand of the same rotifer species: 33.4 vs. $3.4 \text{ mJ} \cdot \text{ind}^{-1} \cdot \text{d}^{-1}$. Based on our observations (Fig. 4) we believe that Doohan's energy budget does not reflect actual relationships in the energetics of *B. plicatilis*. Moreover, she worked at 20°C , where even lower figures than in our experiments were to be expected (cf. Fig. 2). A re-evaluation of the budget on a daily basis should prove worthwhile.

Our estimates of biomass in rotifers and their food (Table 1) can be compared with some data found in the literature: Theilacker & McMaster (1971) determined dry weight (DW; oven-drying at 60°C) and energy content (direct calorimetry) for *B. plicatilis*. Both values (per individual) provided by them are lower than ours (160 vs. 280 ng and 3.3 vs. 4.3 mJ). Also the carbon content measured by us is higher than comparable figures given by Dewey (1976): 116 vs. 90 ng . These differences suggest that, due to our sieving procedure (see above), small individuals were removed from the experimental popula-

tion. Such details of experimental methods must be considered when data from the literature are used for calculations of biomass or energetics.

Our estimate for long-term feeding rate (ca. 7,200 yeast cells or $3.4 \text{ mJ} \cdot \text{ind}^{-1} \cdot \text{d}^{-1}$) means that a rotifer culture with ca. $500 \text{ ind} \cdot \text{ml}^{-1}$ should need an energy supply of ca. $3.9 \cdot 10^9$ cells or $1.7 \text{ kJ} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$ at 23.5°C . This corresponds to ca. 0.1 g DW or 0.5 g fresh weight of yeast. Yúfera & Pascual (1980) found almost identical figures: optimal growth was achieved in cultures at 23°C , when $0.5 \text{ g yeast} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$ were added, however, in combination with algae. Furukawa & Hidaka (1973) estimated far higher ingestion rates for *B. plicatilis* feeding on marine yeast cells ($3.1 \cdot 10^4$ to $4.1 \cdot 10^4 \text{ cells} \cdot \text{ind}^{-1} \cdot \text{d}^{-1}$) and consequently, they added a higher amount ($4 \cdot 10^{10} \text{ cells} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$), when again $500 \text{ ind} \cdot \text{ml}^{-1}$ are taken as an example for rotifer density. They did not provide, however, size, DW, or energy content of their marine yeast, so that direct comparison between these figures for daily food demand is not possible.

Our observations have shown that many methodological details may influence the measurement of filtration and ingestion rates in *B. plicatilis*; hence, reliable comparison of literature data will not be possible until standardized techniques are applied.

Acknowledgements. This research was supported by the Bundesministerium für Forschung und Technologie (Grant No. MFU-0328/1). Dr. W. Greve provided the original rotifer cultures. Food algae and unpublished data on *Dunaliella* spec. were obtained from Dr. E. Hagmeier. Mr. G. Sahling and Mr. J. Ufer helped to operate the Coulter Counter and CHN analyzer. Drawings were made by Mrs. B. Lämmel. Our thanks are also due to Dr. M. Rieper for correcting the manuscript.

LITERATURE CITED

- Capuzzo, J. M., 1979. The effects of halogen toxicants on survival, feeding and egg production of the rotifer *Brachionus plicatilis*. – Estuar. coast. mar. Sci. 8, 307–316.
- Chotiyaputta, C. & Hirayama, K., 1978. Food selectivity of the rotifer *Brachionus plicatilis* feeding on phytoplankton. – Mar. Biol. 45, 105–111.
- Doohan, M., 1973. An energy budget for adult *Brachionus plicatilis* Muller (Rotatoria). – Oecologia 13, 351–362.
- Dewey, J. M., 1976. Rates of feeding, respiration and growth of the rotifer *Brachionus plicatilis* and the dinoflagellate *Noctiluca miliaris* in the laboratory. – Thesis, University of Washington.
- Erman, L. A., 1956. Quantitative aspects of the feeding of rotifers. – Zool. Zh. 35, 965–971.
- Erman, L. A., 1962. On the quantitative aspects of the feeding and food selectivity in the rotifer *Brachionus calyciflorus* Pall. – Zool. Zh. 41, 34–47.
- Furukawa, I. & Hidaka, 1973. Technical problems encountered in the mass culture of the rotifer *Brachionus plicatilis* using marine yeast as food organisms. – Bull. Plankt. Soc. Japan 20, 61–71.
- Galkovskaja, N. Q., 1963. Utilization of food for growth and conditions for maximum production of the rotifer *Brachionus calyciflorus* Pallas. – Zool. Zh. 42, 506–512.
- Gatesoupe, F.-J. & Robin, J. H., 1981. Commercial single-cell proteins either as sole food source or in formulated diets for intensive and continuous production of rotifers (*Brachionus plicatilis*). – Aquaculture 25, 1–15.
- Gatesoupe, F.-J. & Luquet, P., 1981. Practical diet for mass culture of the rotifer *Brachionus plicatilis*: application to larval rearing of sea bass, *Dicentrarchus labrax*. – Aquaculture 22, 149–163.
- Gauld, D. T., 1951. The grazing rate of planktonic copepods. – J. mar. biol. Ass. U. K. 29, 695–706.
- Gilbert, J. J. & Starkweather, P. L., 1977. Feeding in the rotifer *Brachionus calyciflorus*. I. Regulatory mechanisms. – Oecologia 28, 125–131.
- Guillard, R. R. L. & Ryther, P. L., 1962. Studies on marine planktonic diatoms. – Can. J. Microbiol. 8, 229–240.

- Halbach, U. & Halbach-Keup, G., 1974. Quantitative Beziehungen zwischen Phytoplankton und der Populationsdynamik des Rotators *Brachionus calyciflorus* Pallas. Befunde aus Laboratoriumsexperimenten und Freilanduntersuchungen. – Arch. Hydrobiol. 73, 272–309.
- Hargis, J. R., 1977. Comparison of techniques for the measurement of zooplankton filtration rates. – Limnol. Oceanogr. 22, 941–945.
- Hirayama, K. & Ogawa, S., 1972. Fundamental studies on physiology of rotifers for its mass culture. I. Filter feeding rotifer. – Bull. Jap. Soc. scient. Fish. 38, 1207–1214.
- Starkweather, P. L. & Gilbert, J. J., 1977. Feeding in the rotifer *Brachionus calyciflorus*. II. Effect of food density on the feeding rates using *Euglena gracilis* and *Rhodotorula glutinis*. – Oecologia 28, 133–139.
- Starkweather, P. L. & Frost, T. M., 1979. Bacterial feeding by the rotifer *Brachionus calyciflorus*. Clearance and ingestion rates, behaviour and population dynamics. – Oecologia 44, 26–30.
- Theilacker, G. H. & McMaster, M. F., 1971. Mass cultivation of the rotifer *Brachionus plicatilis* and its evaluation as food for larval anchovies. – Mar. Biol. 10, 183–204.
- Yúfera, M. & Pascual, E., 1980. Estudio del rendimiento de cultivos del rotífero *Brachionus plicatilis* O. F. Muller alimentados con levadura de panificación. – Investigación pesq. 44, 361–368.