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FILTRATION AND INGESTION RATES OF THE ROTIFER *BRACHIONUS PLICATILIS* FED FIVE SPECIES OF MICROALGAE AT DIFFERENT CELL DENSITIES

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Abstract

The microalgae *Tetraselmis suecica*, *Nannochloropsis oculata*, *Chlorella* sp., *Isochrysis galbana*, and *Dunaliella tertiolecta* were used as food for the rotifer *Brachionus plicatilis* (L type) raised in laboratory conditions at $25\pm 1^\circ\text{C}$ and 25‰ salinity. Filtration and ingestion rates of the rotifer were determined to study the effects of different microalgae densities and feeding times on the feeding behavior of the starved *B. plicatilis*. The highest filtration (11.5×10^{-4} ml/ind/min) and ingestion (246.99×10^2 cells/ind/min) rates were obtained with *N. oculata*. The filtration rates dropped when the microalgae density rose beyond the optimum level, but ingestion rates varied with algae species. Feeding time significantly influenced these rates: after 60 min, both rates dropped.

Introduction

The euryhaline rotifer, *Brachionus plicatilis*, is essential for intensive culture of marine fish and crustacean larvae in many hatcheries. Rotifers are ideal as a first exogenous food source due to their small size (100-300 μ), slow swimming speed, and ability to stay suspended in the water column. They are relatively easy to culture at high densities and can

be enriched with fatty acids and antibiotics (Lubzens et al. 1989; Fielder et al., 2000; Suantika et al., 2000).

Several species of microalgae have been tried as food for rotifer culture, mainly *Chlorella*, *Nannochloropsis*, *Tetraselmis*, *Isochrysis*, and *Dunaliella*. The list has remained almost unaltered for many years (Hotos, 2003). The effect

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of food particle concentration on the feeding rate of filter-feeding rotifers is an interesting question. Filtration and ingestion rates are among the measurements most closely related to feeding behavior. These rates are influenced by food type, mainly the presence or absence of a cell wall (Hotos, 2003), critical cell density, cell volume (Yufera and Pascual, 1985), type of rotifer (Korstad et al., 1989), and culture conditions (Pourriot, 1977). The use of different cell densities for feeding rotifers (especially cultured *B. plicatilis*) has been studied often but the effects of microalgae density on filtration and ingestion rates are less documented (Pourriot, 1977; Starkweather, 1980; Nagata, 1985; Yufera and Pascual, 1985; Korstad et al., 1989; Hotos, 2002, 2003). It is generally agreed that filtration rates drop and ingestion rates remain approximately constant at high algae density, but it is not clear what happens at low algae density with starved rotifers. The aims of this study were to describe the optimum cell density and feeding behavior of starved *B. plicatilis* exposed to low algae densities by determining the filtration and ingestion rates using five types of microalgae (*Tetraselmis suecica*, *Nannochloropsis oculata*, *Chlorella* sp., *Isochrysis galbana*, and *Dunaliella tertiolecta*).

Materials and Methods

Cultures. *Brachionus plicatilis* (210-320 μ) and the five species of dietary microalgae were cultured at 25°C, 25 ppt salinity, and continuous illumination at 1000 lux. The microalgae were derived from unialgal batch cultures. Algae cells were removed from exponential phase cultures and cell suspensions were diluted with filtered and sterilized sea water to obtain the required concentrations. Before starting the experiment, the rotifers were kept separately at a density of about 100 ind/ml, then starved for 48 h.

Experimental design. In the first stage, a total of 105 (five algae species x seven cell densities x three replicates) Erlenmeyer flasks of 500 ml were used. Each contained 100 ml of the specified algae type and density, as follows: for *T. suecica* 3.2, 6.4, 9.6, 12.8, 16.0, 19.2, 22.4 x 10⁵ cells/ml, for *N. oculata* 60,

120, 180, 240, 300, 360, 420 x 10⁵ cells/ml, for *Chlorella* sp. 16, 32, 48, 64, 80, 96, 112 x 10⁵ cells/ml, for *I. galbana* 17, 34, 51, 68, 85, 102, 119 x 10⁵ cells/ml, and for *D. tertiolecta* 24, 48, 72, 96, 120, 144, 168 x 10⁵ cells/ml. Initial rotifer density was 10 ind/ml in all groups. Algae cell density was counted after 60 min, and maximum filtration and ingestion rates were determined for each microalgae species.

In the second stage, the algae cell density that resulted in the maximum filtration rate for each microalgae during the first stage was continued for an additional 180 min to determine the effect of feeding time on the filtration and ingestion rates, which were calculated at various times from 60 to 240 min.

Filtration and ingestion rates. Filtration and ingestion rates were calculated as follows (Yufera and Pascual, 1985): $F = (\ln C_0 - \ln C_t) / R \times t$, where F = filtration rate in ml/ind/min, C_0 = initial algae density in cells/ml, C_t = final algae density in cells/ml, R = rotifer density in cells/ml, and t = duration of the treatment in min, and $I = F \times \sqrt{C_0 \times C_t}$, I = ingestion rate in consumed algae cells/min. Statistical analyses were performed with SPSS 9.0 for Windows Software (SPSS Inc, Chicago, IL, USA). Data were analyzed by one-way analysis of variance (ANOVA) and multiple comparisons were made with Duncan's multiple range test. The significance level was $p < 0.05$.

Results

The filtration and ingestion rates for all species during the first hour were significantly affected by algae density (Fig. 1). The peak values for both rates in *B. plicatilis* fed *T. suecica* occurred at 16 x 10⁵ cells/ml while for *N. oculata*, the peak filtration rate was 11.5 x 10⁻⁴ ml/ind/min at 180 x 10⁵ cells/ml and the peak ingestion rate was 246.99 x 10² cells/ind/min at 360 x 10⁵ cells/ml. For *Chlorella* sp. and *I. galbana*, well defined peaks for both rates occurred at 80 and 85 x 10⁵ cells/ml, respectively. The peak filtration rate for *D. tertiolecta* occurred at 120 x 10⁵ cells/ml. The ingestion rate decreased at higher densities for each experimental group except *D. tertiolecta*.

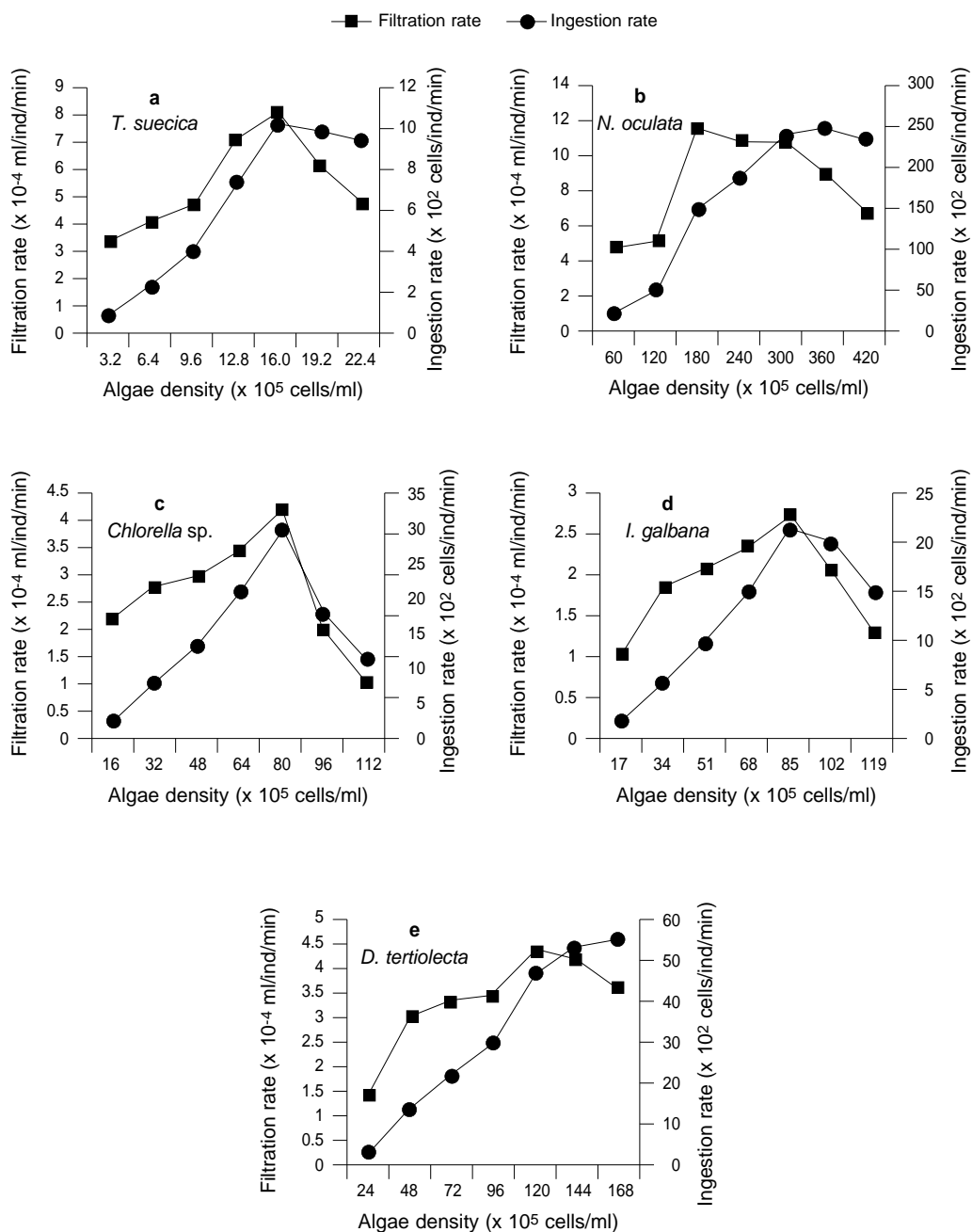


Fig. 1. Filtration and ingestion rates within the first 60 min in the rotifer *Brachionus plicatilis* fed (a) *Tetraselmis suecica*, (b) *Nannochloropsis oculata*, (c) *Chlorella* sp., (d) *Isochrysis galbana*, and (e) *Dunaliella tertiolecta* at different densities.

Filtration and ingestion rates up to 240 min are shown in Fig. 2. Both rates were negatively affected by the feeding time, i.e., the highest rates were obtained during the first 60 min in each group (except for the *N. oculata* ingestion rate reached at 120 min), and then the rates decreased 25-30% within 120 min. Rates at different feeding times differed significantly.

Discussion

In this study, the feeding behavior of *B. plicatilis* with different microalgae species commonly used as food for rotifer culture was investigated. Pourriot (1977) and Starkweather (1980) reported that the filtration rate of rotifers decreased as the algae density increased whereas the ingestion rate increased at lower densities and remained constant at higher densities. In the present study, the filtration rate in each group increased as cell densities increased in low concentrations while it decreased at high densities, except with *D. tertiolecta*. In our study, the highest filtration and ingestion rates were found in *B. plicatilis* fed *N. oculata*; they were 2.5-3 and 8-8.5 times higher than those of *B. plicatilis* fed *Chlorella* (Table 1). The *N. oculata* filtration rate was 4-5, 2.5-3, and 1-2 times higher than those of *I. galbana*, *D. tertiolecta*, and *T. suecica*.

Using *N. oculata*, *N. maculata*, *N. oculata*, and *N. gaditana* of 1.8, 2.1, 2.5, and 2.7 μm mean cell diameters, respectively, at a wide range of densities, Yufera and Pascual (1985) recorded maximum filtration rates of *B. plicatilis* of 1.2×10^{-4} ml/ind/min for the smallest cells and $0.8-1 \times 10^{-5}$ ml/ind/min for the largest. In all four species, the filtration rate decreased following an inverse hyperbolic line as food density increased, maintaining a low plateau beyond a certain high density, whereas the ingestion rate increased almost constantly in a reverse manner. Our filtration rates are in agreement. Our peak filtration rates for each algae except *N. oculata* were higher than theirs but our highest filtration rate (for *N. oculata*) was similar. On the other hand, the ingestion rate for each algae except *D. tertiolecta* dropped as the algae density increased. Their ingestion rate increased to a

peak and then dropped as algal density increased only for *N. gaditana*. This may have been due to the algae species, as observed in the case of ingestion rates in the present study.

Hotos (2002) obtained a maximum filtration rate for *Chlorella* of 25×10^{-5} ml/ind/min at densities of 2.5×10^6 cells/ml; the rate then dropped abruptly and maintained a low plateau at densities over 15×10^6 cells/ml. The ingestion rate followed a similar pattern with values of 150-1400 cells/ind/min. Hotos (2003) obtained peak filtration and ingestion rates with *Chlorella* (22×10^{-5} ml/ind/min and 1200-1400 cells/ind/min, respectively) at densities of $4-8 \times 10^6$ cells/ml for both L and S types of starved rotifers. Our peak filtration and ingestion rates for *Chlorella* occurred at 80×10^5 cells/ml, 1.5-2 times higher than those of Hotos. Vadstein et al. (1993) obtained a peak filtration rate for *Isochrysis* of 24×10^{-5} ml/ind/min. Their results are similar to ours for *Chlorella* and remarkably lower than ours for *I. galbana*.

Inadequacy of available data exists in attempting to evaluate differences between ingestion and filtration rates of rotifers reported in the literature and this study. Differences may be due to differences in the physiological state of the organisms involved with culture conditions, type of rotifer, algae species, and cell density.

Feeding time had a significant effect on the filtration and ingestion rates in this study. Rates at 240 min were much lower than rates at 60 min. This phenomenon was investigated by Schlosser and Anger (1982). Working with *B. plicatilis* in experiments lasting 15-240 min, they observed that the feeding rates at 240 min were 90% lower than the feeding rates at 15 min. For all groups, filtration and ingestion rates decreased after the first hour (about 25-30% in 120 min) and remained approximately constant, as in the present study. Once the gut becomes full, the rate of energy intake is limited by the gut passage time and speed of ingestion (Navarro, 1999; Hotos, 2003). This is probably due to several causes such as a saturation effect with time or stress caused during the experiment which can cause

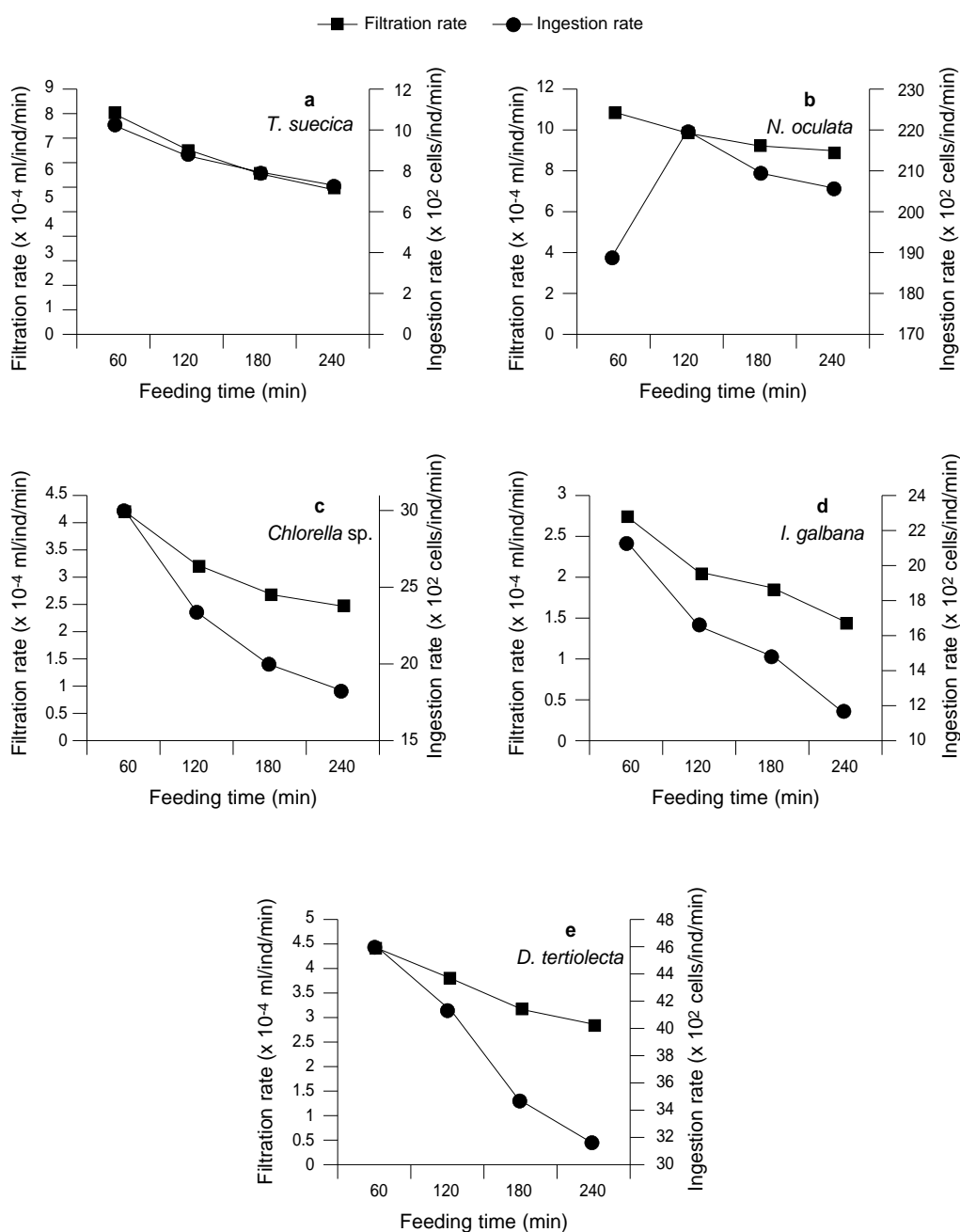


Fig. 2. Filtration and ingestion rates after 60 min and up to 240 min in the rotifer *Brachionus plicatilis* fed (a) *Tetraselmis suecica* at a density of 16×10^5 cells/ml, (b) *Nannochloropsis oculata* at a density of 180×10^5 cells/ml, (c) *Chlorella* sp. at a density of 80×10^5 cells/ml, (d) *Isochrysis galbana* at a density of 85×10^5 cells/ml, and (e) *Dunaliella tertiolecta* at a density of 120×10^5 cells/ml.

Table 1. Maximum filtration and ingestion rates in *Brachionus plicatilis* during and following the first hour of feeding.

Algae species	First 60 minutes		Following three hours	
	Filtration rate (x 10 ⁻⁴ ml/ind/min)	Ingestion rate (x 10 ⁻² cells/ind/min)	Filtration rate (x 10 ⁻⁴ ml/ind/min)	Ingestion rate (x 10 ⁻² cells/ind/min)
<i>Tetraselmis suecica</i>	8.17 ^b	10.23 ^e	6.70 ^b	8.76 ^e
<i>Nannochloropsis oculata</i>	11.5 ^a	246.99 ^a	9.86 ^a	220.03 ^a
<i>Chlorella</i> sp.	4.24 ^d	29.86 ^c	3.20 ^d	23.25 ^c
<i>Isochrysis galbana</i>	2.76 ^e	21.59 ^d	2.08 ^e	16.60 ^d
<i>Dunaliella tertiolecta</i>	4.42 ^c	54.88 ^b	3.89 ^c	41.53 ^b

*Different superscripts in a row indicate significant ($p < 0.05$) differences.

rotifers to throw out their stomach content and feed at higher rates (Navarro, 1999).

In conclusion, the algae species and feeding time affect feeding behavior of rotifers. The filtration rate drops when the algae density increases beyond a certain optimum level and the ingestion rate varies according to microalgae species. The best algae density differs among species. In fish hatcheries, these cell densities can be used to mass-culture algae such as *B. plicatilis*.

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