Indian J. Fish., 63(3): 76-80, 2016
DOI: 10.21077/ijf.2016.63.3.58554-10

Effect of microalgal diets on filtration and ingestion rates of the rotifer *Brachionus plicatilis*

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ABSTRACT

Effect of marine microalgal diet on filtration and ingestion rates of *Brachionus plicatilis* was studied. Marine microalgae *viz*., *Nannochloropsis oculata, Isochrysis galbana, Chaetoceros calcitrans* and a combination of *N. oculata* and *I. galbana* (*Nanno+Iso*) at different cell concentrations were selected for the experiment and triplicates were maintained for each feed. Significant difference ($p<0.05$) was observed in the filtration and ingestion rates of *B. plicatilis,* between the treatments. Peak filtration rate of $12.2x10^{-5}$ cells ml^{-1} ind^{-1} min^{-1} was recorded in *B. plicatilis* fed with *Nanno+Iso,* followed by *I. galbana*. Ingestion rate was found to be significantly high ($p<0.05$) for those fed with *C. calcitrans,* followed by *Nanno+Iso*. Filtration rate was significantly high ($p<0.05$) in rotifers stocked at an initial density of 50 nos. ml^{-1} fed with *Nanno+Iso*. The results indicated that a combination of *Nanno* and *Iso* is the best suitable microalgal diet for rotifer with peak filtration ($12.2x10^{-5}$cells ml^{-1} ind^{-1} min^{-1}) and ingestion ($5.4x10^{-3}$ cells ml^{-1} ind^{-1} min^{-1}) rates during the first 60 min.

Keywords: *Brachionus plicatilis,* Feeding behaviour, Filtration rate, Ingestion rate, Microalgae, Rotifer

Introduction

The rotifer, *Brachionus plicatilis* is one of the most important live feed used in intensive larval rearing of marine finfish in hatcheries. Due to their small size and slow swimming activity, rotifers are popular as starter live feed source for fish larvae. The success of commercial finfish hatcheries mostly depends on the large scale production of live feeds especially rotifers. Several reports are available on the efficacy of different microalgae as feed for rotifer culture (Gopakumar, 1998; Hotos, 2002; Okauchi, 2004; Chew and Lim, 2005; Kostopoulou and Vadstein, 2007; Molly Varghese and Krishnan, 2010; Campana-Torres *et al*., 2012). Few reports are also there on filtration and ingestion rates of rotifers (Schlosser and Anger, 1982; Yufera and Pascual, 1985; Navarro, 1999; Hotos, 2003; Sevgi and Zekiye, 2006) and the studies state that the stocking density significantly impacts the filtration and ingestion rates of rotifers fed with different microalgal diets. Studies on the influence of different microalgal diets in different combinations on filtration and ingestion rates are meagre in India. Hence, the present study was carried out to evaluate the effect of different microalgal diets in different concentrations on the filtration and ingestion rates of *B. plicatilis* stocked at different densities.

Materials and methods

To study the filtration and ingestion rates of the rotifer *B. plicatilis*, three sets of experiments were designed. The experiments were carried out in 500 ml Erlenmeyer flasks and triplicates were maintained for each microalgal species. Four algal feeds selected were *Nannochloropsis oculata, Isochrysis galbana, Chaetoceros calcitrans* and a combination of *N. oculata* and *I. galbana* (*Nanno+Iso*). The cultures were maintained in laboratory at 26ºC at a salinity of 26 ppt with continuous illumination of 1000 lux. Rotifers used for the experiments were starved for 24 h prior to initiation of the experiment. The first experiment was conducted with different concentrations of microalgae @ 2, 8, 12, 22 and $32x10^6$ cells ml^{-1} at a fixed initial rotifer density of 50 no. ml^{-1}. The second experiment was carried out with varying initial densities of *B. plicatilis viz.*, 50, 100, 200 and 500 nos. ml^{-1} with fixed initial algal density of $1x10^6$ cells ml^{-1}. In the third experiment, the density of rotifer and micro algae was fixed at 50 nos. ml^{-1} and $1x10^8$ cells ml^{-1} respectively. Triplicates were maintained for each experiment and the variation in the ingestion and filtration rates of *B. plicatilis* were analysed at different time intervals *viz.,* 60,120,180 and 240 min.

Effect of feeding time on the filtration and ingestion rates were calculated using the formula (Yufera and Pascual, 1985):

$$F = (\ln C_0 - \ln C_t)/R \times t$$

where F = filtration rate (ml^{-1} $ind.^{1}$ $min.^{1}$), C_0 = initial algal density (cells ml^{-1}), C_t = final algae density in cells ml^{-1}, R = rotifer density in cells ml^{-1} and t = duration of the treatment (min.) and

$I = F \times \sqrt{C_0} \times C_t$, I = ingestion rate of consumed algal cells min^{-1}. Variations in density between different diets and between treatments were analysed using one-way analysis of variance (ANOVA).

Results

Experiment 1

The experiment with different cell concentrations of microalgal diets (2, 8, 12, 22 and 32 x 10^6 cells ml^{-1}) and a fixed initial rotifer density (50 nos. ml^{-1}) for the first 60 min revealed that the filtration and ingestion rates of rotifers varied significantly ($p = 0.05$)between the treatments. Peak filtration rate of *B. plicatilis* fed with *N. oculata* was $6.8x10^{-5}$cells ml^{-1} ind^{-1} min^{-1} occurred at cell density of $12x10^6$ cells ml^{-1}. It was observed that the filtration rate of rotifers fed with *N. oculata* was less at lower cell densities of $2x10^6$ cells ml^{-1} and $8x10^6$ cells ml^{-1} and also at higher cell densities of 22 and $32x10^6$ cells ml^{-1}. Ingestion rates also exhibited similar trend and the peak value was recorded at $8x10^6$ cells ml^{-1} (Fig. 1a).

Filtration rates of rotifers fed with *I. galbana* showed higher values at lower cell concentrations with peak value of $6.8x10^{-5}$ cells ml^{-1} ind^{-1} min^{-1} at $2x10^6$ cells ml^{-1}. The lowest filtration rate was recorded at $32x10^6$ cells ml^{-1}. Peak ingestion rate of $0.42x10^{-3}$cells ml^{-1} ind^{-1} min^{-1} was recorded at $2x10^6$ cells ml^{-1} (Fig. 1b). Peak filtration and ingestion rates of rotifers fed with *C. calcitrans* were recorded at cell density of $2x10^6$ cells ml^{-1} and 8 $x10^6$ cells ml^{-1} respectively and the rates were 4.6 x 10^{-5} cells ml^{-1} ind^{-1} min^{-1} and 3.2x 10^{-3} cells ml^{-1} ind^{-1} min^{-1} respectively. It was observed that the rates were lower at higher cell densities (Fig. 1c). The filtration rates of rotifers were found to be less at algal cell densities of 22 $x10^6$ and $32x10^6$ cells ml^{-1} and a peak rate of 12.2 x 10^{-5} cells ml^{-1} ind^{-1} min^{-1} was recorded at $12x10^6$ cells ml^{-1}. Ingestion rates also showed same trend with a peak value of 2.9 $x10^{-3}$ cells ml^{-1} ind^{-1} min^{-1} at $12x10^6$ cells ml^{-1} (Fig. 1 d).

In this experiment, the peak filtration rate was recorded in rotifer fed with *Nanno+Iso* at cell density of $12x10^6$ cells ml^{-1} followed by *I. galbana* and *N. oculata.* Peak ingestion rate was found in rotifer fed with *C. calcitrans* at $8x10^6$ cells ml^{-1}. Peak filtration rates of *N. oculata* and *Nanno+Iso* occurred at the same cell density of $12x10^6$ cells ml^{-1} but the peak ingestion rates varied between the algal diets and cell densities. Peak filtration rates of rotifers fed with *C. calcitrans* and *I. galbana* were found at the same cell densities *i.e.,* $2x10^6$ cells ml^{-1} but peak ingestion rates varied with cell densities of the two species. Out of all the four species, the ingestion rates were very low in rotifer fed with *N. oculata* followed by *I. galbana.* The study also revealed significant variation in the filtration and ingestion

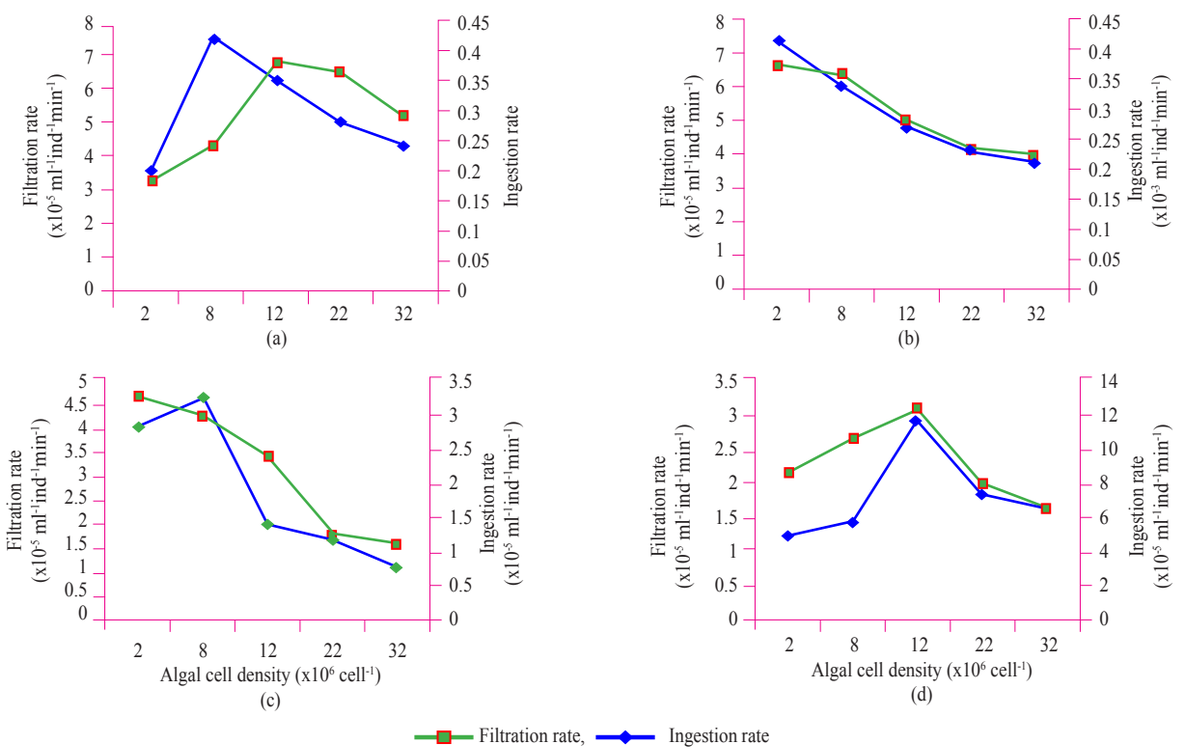

Fig. 1. Filtration and ingestion rates, *B. plicatilis* fed withdifferent cell concentrations of microalgal diets. a: *N. occulata*, b: *I. galbana*, c: *C. calcitrans*, d: *Nanno+Iso*

rates of rotifers between the algal species during the first hour ($p<0.05$, $p=0.00046$).

Experiment 2

The experiment conducted to study the filtration and ingestion rates of rotifers fed with microalgal diets at fixed algal density ($1x10^6$ cell ml^{-1}) and varied initial rotifer densities (50, 100, 200, 500 nos. ml^{-1}), revealed peak filtration and ingestion rates of rotifers at initial rotifer density of 50 nos. ml^{-1}.

Peak filtration rate of rotifers fed with *N. oculata* was $7.2x10^{-5}$ cells ml^{-1} ind^{-1} min^{-1} at initial rotifer density of 50 nos. ml^{-1} and the lowest filtration rate was recorded at 500 nos. ml^{-1}. The ingestion rate was at its peak of $1.5x10^{-3}$ cells ml^{-1} ind^{-1} min^{-1} at 50 nos. ml^{-1} and the lowest rate was $0.5x10^{-3}$ cells ml^{-1} ind^{-1} min^{-1} at 500 nos. ml^{-1}. Filtration and ingestion rates of rotifers fed with *I. galbana* showed peak values at 50 nos. ml^{-1} and the rates were $7.8x10^{-5}$ cells ml^{-1} ind^{-1} min^{-1} and $2.8x10^{-3}$ cells ml^{-1} ind^{-1} min^{-1} respectively. Filtration and ingestion rates of rotifers fed with *C. calcitrans* were maximum at 50 nos. ml^{-1} and ingestion rates were found to be higher compared to filtration rates at initial rotifer densities of 200 and 500 nos. ml^{-1}.

In this study, peak filtration ($8.5x10^{-5}$ cells ml^{-1} ind^{-1} min^{-1}) and ingestion rates ($4.2x10^{-3}$ cells ml^{-1} ind^{-1} min^{-1}) of rotifers were recorded in those rotifers fed with *Nanno+Iso* diet (initial rotifer density of 50 nos. ml^{-1}). Lowest filtration and ingestion rates of $1.6x10^{-5}$ cells ml^{-1} ind^{-1} min^{-1} and $0.5x10^{-3}$ cells ml^{-1} ind^{-1} min^{-1} were observed at the highest initial density of rotifers (500 nos. ml^{-1}) fed with *C. calcitrans* and *N. oculata* respectively (Fig. 2a, b). A significant variation in the filtration and ingestion rates of rotifers with the initial rotifer densities as well as the microalgal diets was observed. Ingestion rates were significantly lower in the batch fed with *N. oculata* ($p<0.05$, $p=0.00092$).

Experiment 3

The experiment conducted with fixed microalgal density as well as initial rotifer density showed peak filtration rate of rotifer with microalgal diet of *Nanno+Iso* combination followed by *N. oculata, I. galbana* and *C. calcitrans* during the first 60 min. Filtration rate decreased gradually from 60 min to 240 min of feeding time for all the diets.

Peak filtration and ingestion rates of *N. occulata* were recorded as $8.6x10^{-5}$ cells ml^{-1} ind^{-1} min^{-1} and 3.6×10^{-3} cells ml^{-1} ind^{-1} min^{-1} at first 60 min, whereas for other diets *viz., I. galbana., C. calcitrans* and *Nanno+Iso,* the peak filtration rates were 7.8, 6.5 and $10.2x10^{-5}$ cells ml^{-1} ind^{-1} min^{-1} respectively (Fig. 3a-d). Ingestion rates of all the diets followed the same trend and declined with time (Fig. 3b). Filtration and ingestion rates of rotifers with fixed algal concentration and fixed initial rotifer density varied significantly between the algal diets as well as with duration of time ($p<0.05$). Ingestion rate of rotifers decreased significantly with duration of time from 4.2×10^{-3} cells ml^{-1} ind^{-1} min^{-1} (60 min) to $0.8x10^{-3}$ cells ml^{-1} $ind^{-1}min^{-1}$ (240 min) when fed with *I. galbana.*

Results of the present study revealed that cell densities of microalgal diets and the feeding time played a significant role on filtration and ingestion rates of rotifers. It was observed that the filtration rates were high in all the three experiments when fed with a combination diet of *Nanno+Iso* during the first 60 min. It was also noted that the filtration rates of *C. calcitrans* and *I. galbana* dropped when the cell densities were high whereas, the rates were high initially up to 12×10^6 cells ml^{-1} for those fed with *N. oculata* and a combination of *Nanno+Iso.* Both filtration and ingestion rates were dropped with time from 60 min to 240 min. In all the three experiments, a significant variation in filtration and ingestion rates of rotifers between all the treatments was evident ($p<0.05$, $p=0.0014$).

Discussion

The present study showed that the concentration and densities of microalgal diets and initial rotifer density played a major role in the filtration and ingestion rates of rotifers

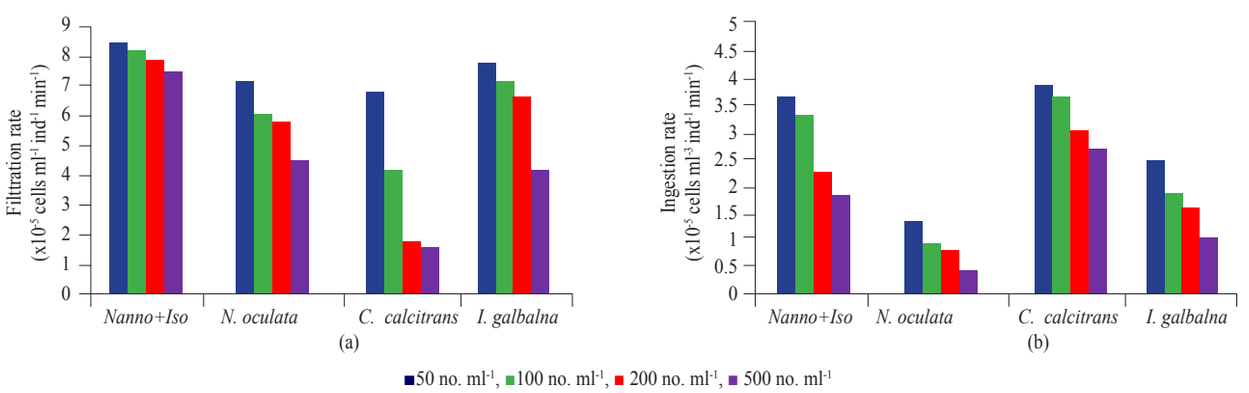

Fig. 2.a. Filtration and b. ingestion rates of *B. plicatilis* fed with different microalgal diets at different initial rotifer densities

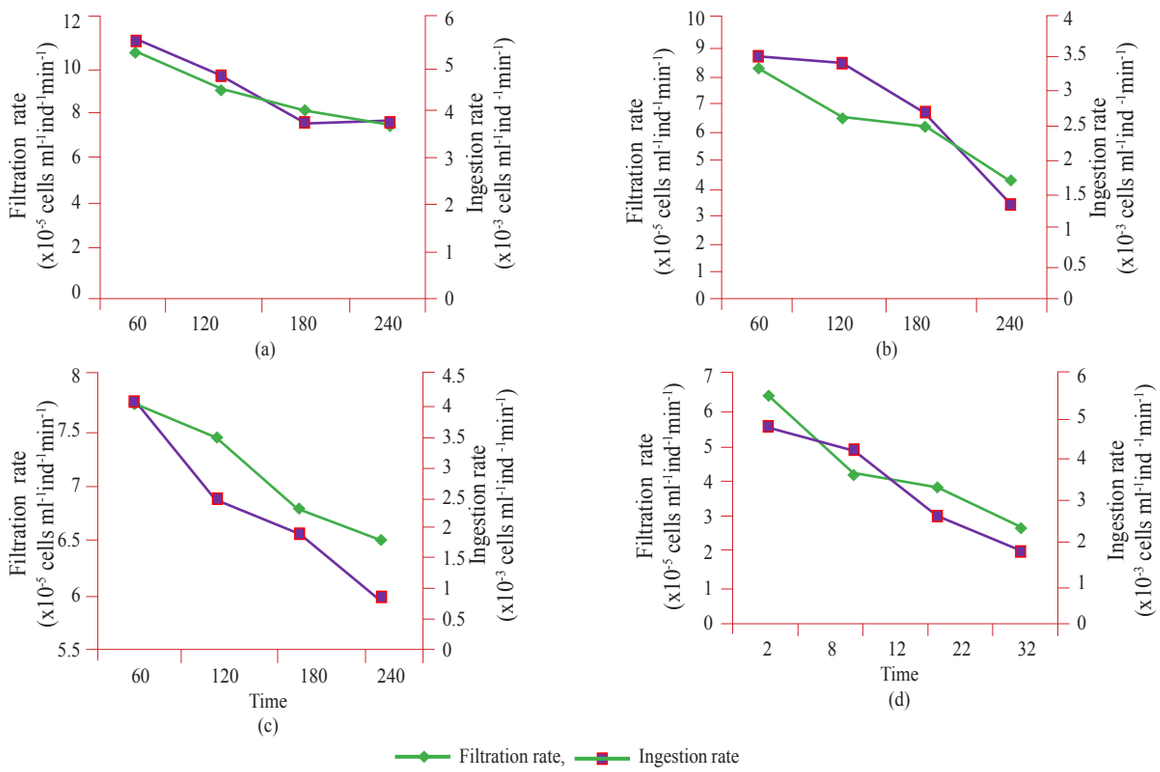

Fig. 3. Filtration and ingestion rates of *B. plicatilis* fed with different microalgal diets with fixed cell concentration (1x108 cells ml^{-1}) and initial rotifer densities (50 number ml^{-1}). a. *Nanno+Iso, b. N. oculata, c. I. galbana, d. C. calcitrans*

and highest rates were recorded during the first 60 min time. Sevgi and Zekiye (2006) reported that the feeding time significantly influenced the ingestion rates *i.e.,* the highest rates were obtained during the first 60 min, except for *Nannochloropsis*.

Peak filtration rate of $12.2x10^{-5}$ cells ml^{-1} ind^{-1} min^{-1} was recorded in *B. plicatilis* fed *Nanno+Iso* followed by *I. galbana*. It was also recorded that the peak values varied with the cell densities of the microalgae and peak filtration occurred at $12x10^6$ cells ml^{-1} for *Nanno+Iso* and *N. oculata*. But the peaks were at $2x10^6$ cells ml^{-1} for *I. galbana* and *C. calcitrans*. The filtration values of the rotifers fed with a *Nanno+Iso* were 2.7 and 1.7 times higher than those of *C. calcitrans* and *I. galbana*. Savas and Zekiye (2006) reported the highest filtration and ingestion rates in *B. plicatilis* fed *N. oculata*. They also reported that the values were 1-2 times higher than those of *I. galbana*. Vadstein *et al*. (1993) observed peak filtration rate of $24x10^{-5}$ cells ml^{-1} ind^{-1} min^1 for *Isochrysis*. But our results showed that filtration rate of rotifer fed with *Nanno* and *Iso* was 3 times higher than that of *I. galbana* and peak filtration was obtained with a combination of *Nanno* and *Iso* at $22x10^6$ cells ml^{-1}. Yufera and Pascual (1985) recorded maximum filtration rates of *B. plicatilis* of $1\text{-}2x10^{-4}$ cells ml^{-1} ind^{-1} min^{-1} for the smallest cells and $0.8\text{-}1x10^{-5}$ cells ml^{-1} ind^{-1} min^{-1} for the largest. Variations in the filtration and ingestion rates of rotifers observed could be attributed to the physiological differences in the organisms fed with different sizes of microalgal diets as well as gut passage time and speed of ingestion (Hotos, 2003).

Pourriot (1977) observed decreased filtration rate in rotifers with increase in cell concentration. In the present study, the filtration and ingestion rates showed a gradual decline with increase in cell density of *C. calcitrans* and *I. galbana*. But filtration rates for rotifer fed with *Nanno+Iso,* were high at concentrations of $2x10^6$ cells ml^{-1}, $8x10^6$ cells ml^{-1} and $12x10^6$ cells ml^{-1} and the rates declined at cell concentrations of $22x10^6$ cells ml^{-1} and $32x10^6$ cells ml^{-1}.Hotos (2003) reported an abrupt drop in filtration and ingestion rates with increase in cell density of *Chlorella* sp.

The present study on the impact of varying cell densities of microalgae and initial rotifer densities on filtration rates of rotifers revealed that a combination of *Nanno* and *Iso* is the ideal microalgal diet with peak filtration and ingestion of rotifers in the first 60 min in all the three experiments compared to those fed with single microalgal diet. It was also observed that cell density of $1.2x10^7$ cells ml^{-1} and initial rotifer density of 50 nos. are the most ideal densities to attain

peak filtration rate and ingestion rates. It was also observed that the cell density in association with duration of time to be consumed by the rotifers had a major impact in enhancing the filtration and ingestion rates. In conclusion, it can be stated that microalgal density and initial rotifer density play major role in feeding behaviour of rotifers and it is suggested that a combination of *N. oculata and I. galbana* is the most suitable microalgal diet for *B. plicatilis*.

Acknowledgments

Authors are thankful to the Director, ICAR-Central Marine Fisheries Research Institute, Kochi for providing facilities to carry out this work.

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Date of Receipt : 22.05.2016
Date of Acceptance : 02.07.2016