



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

JAYASREE LOKA, K. K. PHILIPOSE AND S. M. SONALI

Karwar Research Centre of ICAR-Central Marine Fisheries Research Institute, Karwar - 581 301, Uttarkannada, Karnataka, India

e-mail: lokasree@gmail.com

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 12.2×10^{-5} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ 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 (12.2×10^{-5} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$) and ingestion (5.4×10^{-3} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$) 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 26°C 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 32×10^6 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 1×10^6 cells ml^{-1} . In the third experiment, the density of rotifer and micro algae was fixed at 50 nos. ml^{-1} and 1×10^8 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 ($\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$), 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

$I = F \times \sqrt{C_0 \times C_p}$, 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 $\times 10^6$ 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 6.8×10^{-5} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ occurred at cell density of 12×10^6 cells ml^{-1} . It was observed that the filtration rate of rotifers fed with *N. oculata* was less at lower cell densities of 2×10^6 cells ml^{-1} and 8×10^6 cells ml^{-1} and also at higher cell densities of 22 and 32×10^6 cells ml^{-1} . Ingestion rates also exhibited similar trend and the peak value was recorded at 8×10^6 cells ml^{-1} (Fig. 1a).

Filtration rates of rotifers fed with *I. galbana* showed higher values at lower cell concentrations with peak value of 6.8×10^{-5} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ at 2×10^6 cells ml^{-1} . The lowest filtration rate was recorded at 32×10^6 cells ml^{-1} . Peak ingestion rate of 0.42×10^{-3} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ was recorded

at 2×10^6 cells ml^{-1} (Fig. 1b). Peak filtration and ingestion rates of rotifers fed with *C. calcitrans* were recorded at cell density of 2×10^6 cells ml^{-1} and 8×10^6 cells ml^{-1} respectively and the rates were 4.6×10^{-5} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ and 3.2×10^{-3} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ 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 22×10^6 and 32×10^6 cells ml^{-1} and a peak rate of 12.2×10^{-5} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ was recorded at 12×10^6 cells ml^{-1} . Ingestion rates also showed same trend with a peak value of 2.9×10^{-3} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ at 12×10^6 cells ml^{-1} (Fig. 1d).

In this experiment, the peak filtration rate was recorded in rotifer fed with *Nanno+Iso* at cell density of 12×10^6 cells ml^{-1} followed by *I. galbana* and *N. oculata*. Peak ingestion rate was found in rotifer fed with *C. calcitrans* at 8×10^6 cells ml^{-1} . Peak filtration rates of *N. oculata* and *Nanno+Iso* occurred at the same cell density of 12×10^6 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.*, 2×10^6 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

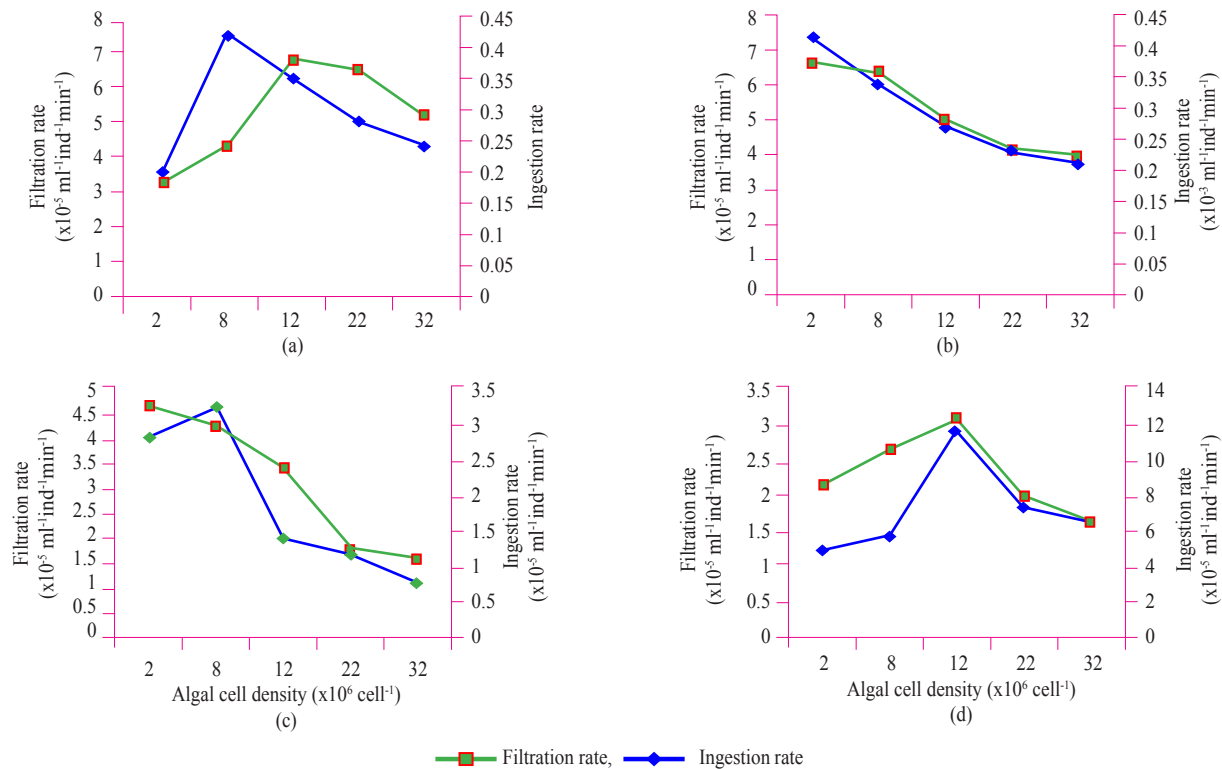


Fig. 1. Filtration and ingestion rates, *B. plicatilis* fed with different cell concentrations of microalgal diets. a: *N. oculata*, b: *I. galbana*, c: *C. calcitrans*, d: *Nanno+Iso*

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 (1×10^6 cells 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 7.2×10^{-5} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ 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 1.5×10^{-3} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ at 50 nos. ml^{-1} and the lowest rate was 0.5×10^{-3} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ 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 7.8×10^{-5} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ and 2.8×10^{-3} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ 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 (8.5×10^{-5} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$) and ingestion rates (4.2×10^{-3} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$) 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 1.6×10^{-5} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ and 0.5×10^{-3} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ 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. oculata* were recorded as 8.6×10^{-5} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ and 3.6×10^{-3} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ 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 10.2×10^{-5} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ 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 $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ (60 min) to 0.8×10^{-3} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ (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

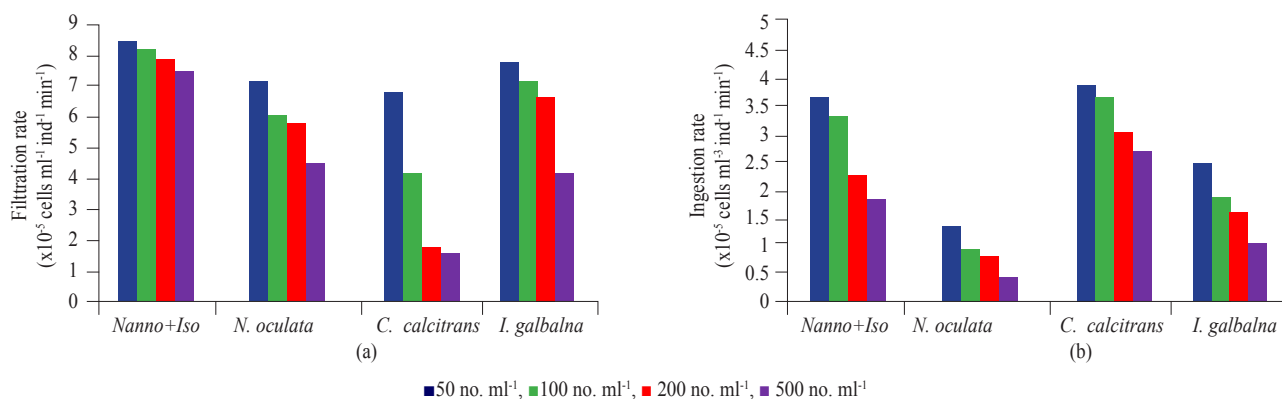


Fig. 2.a. Filtration and b. ingestion rates of *B. plicatilis* fed with different microalgal diets at different initial rotifer densities

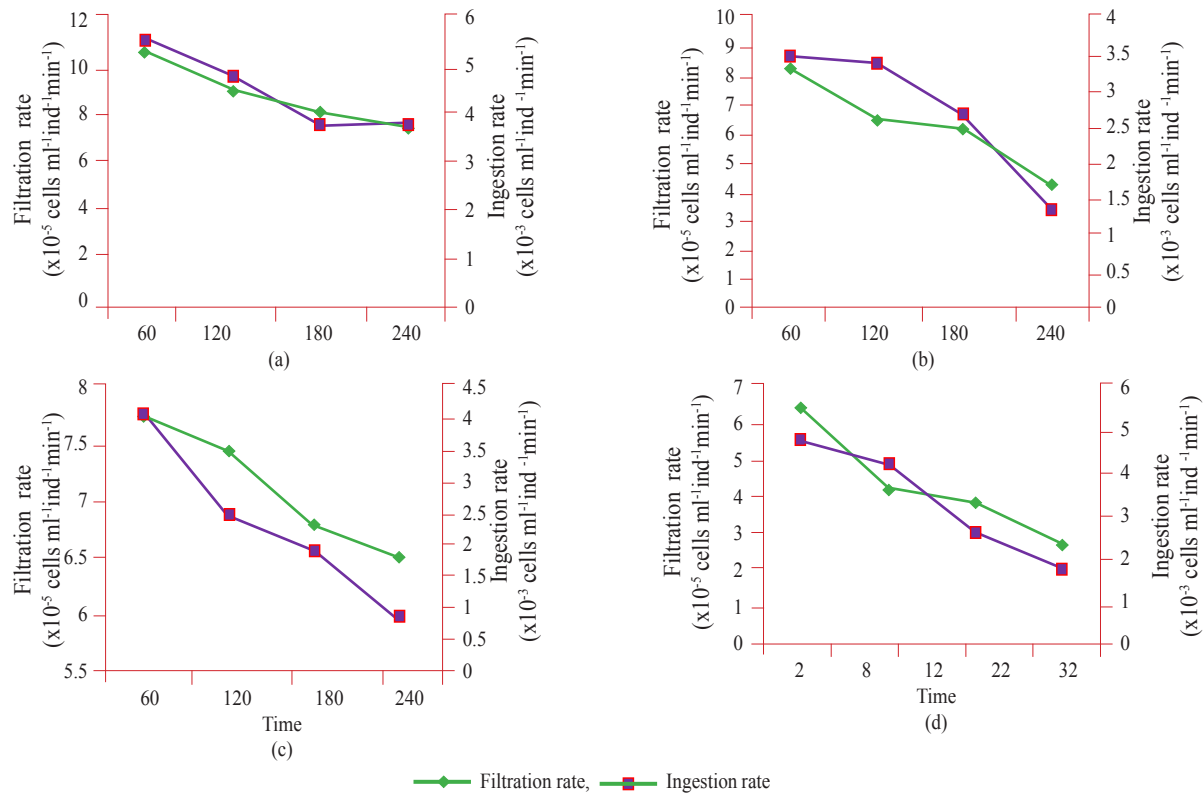


Fig. 3. Filtration and ingestion rates of *B. plicatilis* fed with different microalgal diets with fixed cell concentration (1×10^8 cells ml^{-1}) and initial rotifer densities (50 number ml^{-1}). a. *Nanno*+*Iso*, b. *N. oculata*, c. *I. galbana*, d. *C. calcitrans*

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 12.2×10^{-5} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ 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 12×10^6 cells ml^{-1} for *Nanno*+*Iso* and *N. oculata*. But the peaks were at 2×10^6 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 24×10^{-5} cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ 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 22×10^6 cells ml^{-1} . Yufera and Pascual (1985) recorded maximum filtration rates of *B. plicatilis* of $1-2 \times 10^{-4}$ cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ for the smallest

cells and $0.8-1 \times 10^{-5}$ cells $\text{ml}^{-1} \text{ind}^{-1} \text{min}^{-1}$ 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 2×10^6 cells ml^{-1} , 8×10^6 cells ml^{-1} and 12×10^6 cells ml^{-1} and the rates declined at cell concentrations of 22×10^6 cells ml^{-1} and 32×10^6 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 1.2×10^7 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.

References

- Campana-Torres A., Martinez-Cordova, L. R., Martinez-Porchas, M., Lopez-Elias, J. A. and Porchas-Cornejo, M. A. 2012. Productive response of *Nannochloropsis oculata*, cultured in different media and their efficiency as food for the rotifer *Brachionus rotundiformis*. *Int. J. Exp. Bot.*, 81:44-50.
- Chew, W. Y. S and Lim, H. S. 2005. Some improvements to the rotifer (*Brachionus rotundiformis*) mass culture method. *Singapore J. Pri. Ind.*, 32: 52-58.
- Gopakumar, G. 1998. *Studies on brackishwater rotifers of Kerala with special reference to Brachionus plicatilis O. F. Muller as live feed for aquaculture*. Ph. D. Thesis, University of Kerala. Thiruvananthapuram.
- Hotos, G. N. 2002. Selectivity of the rotifer *Brachionus plicatilis* fed mixtures of algal species with various cell volumes and cell densities. *Aquac. Res.*, 33: 949-957.
- Hotos, G. N. 2003. Growth, filtration and ingestion rate of the rotifer *Brachionus plicatilis* fed with large (*Asteromonas gracilis*) and small (*Chlorella* sp.) celled algal species. *Aquac. Res.*, 34: 793-802.
- Kostopoulou, V. and Vadstein, O. 2007. Growth performance of the rotifers *Brachionus plicatilis*, B. 'Nevada' and B. 'Cayman' under different food concentrations. *Aquaculture*, 273: 449-458.
- Molly Varghese and Krishnan, L. 2010. Reproductive potential of the rotifer, *Brachionus rotundiformis* Tschugunoff in relation to salinity, feed type and feed concentration *Indian J. Fish.*, 57(1): 31-37.
- Navarro, N. 1999. Feeding behaviour of rotifers *Brachionus plicatilis* and *Brachionus rotundiformis* with two types of food: live and freeze-dried microalgae. *J. Exp. Mar. Biol. Ecol.*, 237(1): 75-87.
- Okauchi, M. 2004. An assessment of the beneficial roles of *Nannochloropsis oculata* in larval rearing of marine finfish. *Bull. Fish. Res. Agen. Suppl. No. 1*: 83-90.
- Pourriot, R. 1977. Food and feeding habits of rotifers. *Arch. Hydrobiol.*, 8: 243-260.
- Schlosser, H. J. and Anger, K. 1982. The significance of some methodological effects on filtration and ingestion rates of the rotifer *Brachionus plicatilis*. *Helgolander Meeresunters*, 35: 215-225.
- Sevgi, S. and Zekiye, G. 2006. Filtration and ingestion rates of the rotifer, *Brachionus plicatilis* fed five species of microalgae at different cell densities. *Isr. J. Aquac. Bamidgeh*, 58(1): 39-45.
- Vadstein, O., Gunvor, Q. and Olsen, Y. 1993. Particle size dependent feeding by the rotifer *Brachionus plicatilis*. *Hydrobiologia*, 225-226: 261-267.
- Yufera, M. and Pascual, E. 1985. Effects of algal food concentration on feeding and ingestion rates of *Brachionus plicatilis* mass culture. *Hydrobiologia*, 122: 181-187.