Journal of Applied Phycology
https://doi.org/10.1007/s10811-024-03302-1

RESEARCH

# Effects on fatty acids, biochemical composition and growth of rotifer (*Brachionus plicatilis*) fed with different concentrations of *Nannochloropsis* sp.

**Mustafa İbrahim Osmanoğlu**[1] · **Mahmut Elp**[2] · **Yaşar Durmaz**[3]

Received: 26 December 2023 / Revised: 7 June 2024 / Accepted: 13 June 2024


**Abstract**
The density of microalgae and nutrient content have impact on the population growth rate, egg rate and nutritional values of rotifers. The aim of this study was to investigate the effects of different concentrations of the alga *Nannochloropsis* sp. (N10: $10x10^6$, N20: $20x10^6$, N30: $30x10^6$ cells $mL^{-1}$) on the growth, egg rate, biochemical composition and fatty acid profile of the rotifer *Brachionus plicatilis*. To maintain a consistent algae density, an equivalent quantity was supplemented to compensate for any reduction. The study was started with three replicates, each with an initial inoculation density of 200 *Brachionus* individuals $mL^{-1}$ . The investigation spanned 5 days, during which population parameters (total number of individuals, proportion of individuals with eggs and growth rate) were calculated. After the study period, rotifers were harvested for subsequent biochemical and fatty acid analyses. Notably, the N30 group showed the highest proportions of crude protein (54.41%) and crude lipid (34.5%). The N20 group displayed the most substantial content of total fatty acids, recording a value of 70.69%. Consequently, the N30 group, with a concentration of $30x10^6$ cells $mL^{-1}$, emerged as the most proficient group, demonstrating superior performance in both population growth and biochemical composition.

**Keywords** Rotifer · *Brachionus plicatilis* · Microalgae density · Growth rate · Egg ratio · Biochemical composition · Fatty acids

## Introduction

The critical factors of aquaculture always rely on dietary sources. For this reason, the primary focus of aquaculture needs has consistently been on feed resources (Vidhya et al. 2014). Among these resources, those rich in essential nutrients, like plankton, hold particular importance as they represent the foundational input for the entire aquaculture sector. Live foods, particularly microalgae and zooplankton, are recognized as crucial for larval development in the aquaculture industry (Ramlee et al. 2021). Zooplankton is an essential natural food source for the development of various aquaculture species. Certain fish species, especially larvae and juveniles, primarily derive their nutrients from zooplankton due to their suitability for mouth size and high nutritional value. Zooplankton provides not only nutritious food but also essential substances like proteolytic enzymes, hormones, and biochemicals that facilitate intestinal digestion in the larvae (Grubišić et al. 2012). When culturing zooplankton, microalgae prove to be a suitable choice as they offer a nutrient profile that supports the growth, survival, and life cycle parameters of aquatic filter-feeding organisms, including various zooplankton (Rasdi et al. 2020).

The success of aquaculture depends on healthy culture stock. The way to achieve this is to feed the larvae with live food. Artificial larval feeds cannot compete with live food organisms in terms of acceptance, nutrition and other factors. Additive artificial feeds cannot meet all the elements required for the growth of the fish. Therefore, fish and shellfish need to be fed with live food (Das et al 2012). The use of zooplankton in aquaculture provides significant

This article was part of PhD Thesis of Mustafa İbrahim OSMANOĞLU.

✉ Mustafa İbrahim Osmanoğlu
miosmanoglu@gmail.com

[1] Faculty of Engineering and Architecture, Department of Genetics and Bioengineering, Kastamonu University, Kastamonu, Türkiye

[2] Rafet Vergili Vocational School, Department of Forestry, Kastamonu University, Kastamonu, Türkiye

[3] Aquaculture Department, Faculty of Fisheries, Ege University, İzmir, Türkiye

Published online: 01 July 2024

advantages, especially in larval rearing. Zooplankton is a rich source of protein, fat, vitamins and minerals which are necessary for the rapid growth and healthy development of the larvae, to the larvae. Since zooplanton provides a nutrition similar to the food consumed by the larvae in their natural habitat, it ensures a healthier and more natural development of the larvae. Zooplankton has high digestibility (Ramlee et al. 2021) and can be easily digested by the larvaethereforeensuring more efficient use of nutrients and increasing the growth rate of the larvae (Jeffs and O'Rorke, 2020). Additionally, zooplankton species have antimicrobial properties, making the larvae more resistant to diseases (Woods et al. 2022). The lively nature of zooplankton helps the larvae improve their hunting skills. This improves the larvae's hunting behavior and enables them to search for prey (Martinez-Silva et al. 2018). For such reasons, live food is indispensable for larval culture.

Rotifers (*Brachionus plicatilis*) are the most commonly used live food in commercial marine fish hatcheries. Their suitability largely hinges on the sizes of the larval mouth (Rainuzzo et al. 1997; Reitan et al. 1997; Conceição et al. 2010; Hawkyard et al. 2016). Rotifers remain indispensable in larval culture due to their convenient size and relatively straightforward production techniques. However, the nutritional quality and consistent production of rotifers are heavily reliant on the specific microalgae species employed to feed them. Ensuring the stable production of high-nutrient rotifers is paramount for commercial marine fish hatcheries (Hamre et al. 2013).

The use of fresh microalgae remains a widely employed practice in hatcheries, primarily due to its positive effects on water quality and its ability to enhance the nutrient content of rotifer cultures (Hoff and Snell 2001; Pérez-Legaspi et al. 2018). In marine fish hatcheries producing larval fish, *Nannochloropsis* sp. is the preferred microalgal choice for feeding rotifers. This is attributed to its ease of cultivation, high density, and abundant eicorapentaenoic acid content (Yoshimatsu and Hossain, 2014; Özçiçek et al. 2017).

There exists a highly significant relationship between the reproduction rate of rotifers and the abundance of their food sources. The density of microalgae and nutrient content have a profound impact on the population growth rate, egg rate, and nutritional values,parameters of rotifers (Suchar and Chigbu 2006; Sales et al. 2022). Although studies involving the feeding of *Nannochloropsis* sp. to rotifers have been conducted, research examining the effects of different concentrations of this microalga on culture performance and biochemical composition remains limited. Therefore, this study aimed to determine the effect of different concentrations of *Nannochloropsis* sp. on the growth and biochemical composition of rotifer (*B. plicatilis*).

## Materıals and methods

### Microalgae culture

*Nannochloropsis* sp. was obtained from the Mediterranean Fisheries Research, Production and Training Institute (Antalya/Türkiye). Microalgae were identified according to Algaebase (Guiry and Guiry 2020). Microalgae were cultured in sterile f/2 medium (Guillard and Ryther 1962) indoors at 25 °C, with a salinity of 30 ppt and subjected to continuous light (200 µmol photone $m^{-2}s^{-1}$) for 24 hours. Subcultures were grown in bags, transitioning to culture volumes of 500 mL, 1 L, 5 L, and finally 200 L. When cultivating large volumes of microalgae, seawater was filtered and treated with 5 mg $L^{-1}$ of sodium hypochlorite for 24 h (Agh and Sorgeloos 2005). Subsequently, the sodium hypochlorite was neutralized using sodium thiosulfate (10 mg $L^{-1}$). The microalgal growth rate was determined daily using a Neubauer chamber (Marienfeld-superior). Before starting the study the microalgae were centrifuged at 4000 rpm for 10 min Ahmad et al. 2014). Centrifugation was preferred due to its maximum biomass collection efficiency without causing microalgae cell damage (Sales et al. 2022).

### Rotifer stock culture

*Brachionus plicatilis* (100-210 µm, S type) was obtained from the Mediterranean Fisheries Research, Production, and Training Institute (Antalya/Türkiye). The culture was maintained in a 300 L cylindrical culture vessel at 25 ppt salinity, 5 ppm dissolved oxygen, and 25 °C. The entire culture was renewed every 3 or 5 days. Rotifers were filtered using 60 µm plankton net, rinsed, and transferred to a new culture vessel. Stock culture was fed with baker's yeast (*Saccharomyces cerevisiae*).

### Experiment plan

Before the study, the effect of *Nannochloropsis* sp. on the *B. plicatilis* population was examined at three different concentrations N10: $10x10^6$; N20: $20x10^6$; N30: $30x10^6$ cells $mL^{-1}$. The study was carried out in triplicate. Nine transparent 20-L PET cylindrical containers were utilized. The rotifers were filtered from the stock culture with a plankton net (60 µm) and subsequently transferred to a graduated transfer container containing culture water (salinity of 25 ‰, 25°C). A 1 mL sample was then taken from the transfer container and counted under the microscope in a Sedgewick rafter

counting chamber (Marienfeld-superior). Once the requisite number of rotifers had been transferred to the graduated culture container, the transfer container was shaken slightly to ensure homogeneous distribution of the rotifers. Subsequently, a 1 mL sample was taken from the trial vessel for control purposes and counted under the microscope. Each trial vessel was then adjusted to contain initial rotifer density of 200 individuals per mL. All experiment tanks were continuosly aerates with a ring system. Resun air 8000 (Air flow 540 L $h^{-1}$, pressure ≥ 0.14Mpa) was used as air blower. After the study started, the algal cells in the trial tanks were counted every 24 h and a decreasing number of cells was added to the trial tanks to keep the cell density constant. The experiment spanned 5 days and was carried out at 25°C with a salinity of 25 ‰. Culture containers were closed to allow air in and out to prevent water evaporation. Thus, salinity was kept constant throughout the experiment. Dissolved oxygen (4.6-5 mg $L^{-1}$) and pH (7.8-8.1) varied throughout the experiment.

## Determination of population parameters and counting

The main life history factors of rotifer are population density, instantaneous growth rate and egg rate (Sunil et al 2024). For the estimation of growth parameters, a 1 mL sample was extracted from each group using a pipette in three repetitions at 24-h intervals throughout the experiment. Subsequently, after fixing the rotifers with 2-3 drops of Lugol's solution in the Sedgewick Rafter counting chamber, rotifers, individuals with eggs and amictic eggs responsible for population growth were enumerated under a microscope (ZEISS Stemi 508 Stereo). The growth rate and egg ratio was calculated using the formulae below. Egg ratio refers to the ratio of the total number of eggs in a population to the number of amitic females (Edmondson 1960; Theilacker and McMaster 1971; James and Rezeq 1988).

$$\text{Population density} : \frac{\text{Number of rotifers}}{\text{mL}} \tag{1}$$

$$\text{Instantaneous Growth rate} : r = \frac{In\, N_t - In\, N_o}{t} \tag{2}$$

$$\text{Egg ratio} : \text{ER} = \frac{\text{Number of eggs (ind.mL}^{-1})}{\text{Number of rotifers (ind.mL}^{-1})} \tag{3}$$

where $r$ instantaneous growth rate (divisions $day^{-1}$); $N_o$ initial rotifer density (individuals $mL^{-1}$); $N_t$ maximum rotifer density (individuals $mL^{-1}$) reached after $t$ days; and $t$ number of days to reach maximum individuals $mL^{-1}$.

## Harvesting and sampling procedures

A 100 mL volume of the rotifer culture was transferred onto Whatman GF/C filter paper (47 mm, 1.2 µm), washed with sterile distilled water, and then dried in an oven at 60°C for 24 h. The dry weight was determined by subtracting the weight of the previously tared filter paper from that of the dried samples (Coutinho et al. 2020). As per the study's designed setup, rotifers in each experimental group were harvested by filtration using a 60 µm plankton net. Subsequently, they were washed with sterile distilled water and stored in the refrigerator at -18°C until analysis.

## Proximate composition and fatty acid analysis

Crude protein (AOAC 2010), carbohydrate (Lowry et al. 1951) and crude lipid along with fatty acids (Folch et al. 1957) of both rotifer and microalgae were analyzed. Chloroform/methanol (2:1) solution was prepared for the extraction of lipids. The rotifer sample was placed in a glass tube, the prepared chloroform/methanol solution was poured on it and 200 µL of potassium chloride solution (0.88%) was added. Afterwards, oxidation was prevented with nitrogen. The tubes were capped and shaken. The lower phase in the samples was collected in a tare bottle. The samples were evaporated in an oven and weighed. Lipid was calculated as a percentage according to the formula below (Folch et al. 1957).

$$\%\ \text{Crude Lipid} = \text{Weight change of beaker (g)}/\text{Sample weight (g)} \times 100 \tag{4}$$

For the analysis of fatty acid methyl ester (FAME), samples obtained from crude lipid analysis were used. After evaporating the fatty acid methyl ester under nitrogen solvent, a bortrifluoride solution (containing 10% methanol) was prepared. This solution catalyzed the transesterification of lipid extracts at 92°C. Hexane-dissolved FAME was then subjected to analysis at the Central Research Laboratory using gas chromatography using a GCMS –QP 2010 Ultra, Shimadzu flame ionization detector (GC-FID), and SGE column (30 m x 0.25 mm x 0.25 µm film thickness). Helium served as the carrier gas, with a flow rate set to 1.0 mL $min^{-1}$. The column temperature was initially set at 180°C and increased by 5°C $min^{-1}$ until reaching 220°C. The injector temperature was maintained at 250°C, and the detector temperature at 270°C. The results were compared with a standard lipid solution using the flame ionization detector (FID) (Kates 1986).

## Statistical analysis

The results obtained are presented as the standard deviation of the mean. Statistical analyses were evaluated

using the IBM Statistical Package for Social Science 22.0 (SPSS, USA) program. In the analysis of the dataset, one-way analysis of variance (one-way ANOVA), homogeneity test, and Duncan Multiple Comparison Technique were used to discern the source of the differences between groups. The statistical significance level was set as $p<0.05$.

## Results

### Determination of microalgae concentration

Before starting the study *Nannochloropsis* sp. concentrations that gave the lowest and highest rotifer populations were determined. No eggs were observed in rotifers fed below $10x10^6$ cells $mL^{-1}$ concentration due to lack of food. No significant difference was observed in terms of culture performance in rotifers fed at concentrations of $30x10^6$-$40x10^6$ cells $mL^{-1}$. In those fed at a concentration above $45x10^6$ cells $mL^{-1}$, the lifespan of the rotifers did not exceed 3 days. According to these results, three different *Nannochloropsis* sp. concentrations (N10: $10x10^6$; N20: $20x10^6$; N30: $30x10^6$ cells $mL^{-1}$) were determined.

### Population parameters

The highest number of individuals, along with the number and proportion of individuals with eggs and the growth rate, were recorded on the 4th day in the N30 group. The peak number of individuals reached 1047±20.90 ind. $mL^{-1}$, with the number and proportion of rotifers with eggs at 555±9.0 ind. $mL^{-1}$, accounting for 53±1.9% of the population, and a growth rate of 0.41±0.01 divisions $day^{-1}$ (Fig. 1). This trend was followed by the N20 and N10 groups, respectively. There was a proportional increase in microalgae consumption with the rise in the number of individuals. The highest consumption, calculated on day 4, was $2,880±98x10^4$ cells $mL^{-1}$in the N30 group. The amount of algae consumed throughout the study is shown in Fig. 2.

### Proximate and fatty acid analysis

At the end of the study, following the harvest of rotifers, the biochemical analysis revealed that the highest nutritional values (crude protein, crude lipid and ash) were observed in the N30 group in rotifers fed with different concentrations of *Nannochloropsis* sp. A statistically significant difference was seen in crude lipid only in the N10 group. The carbohydrate content was highest in the N20 group and the statistical difference was found in the N30 group (Table 1).

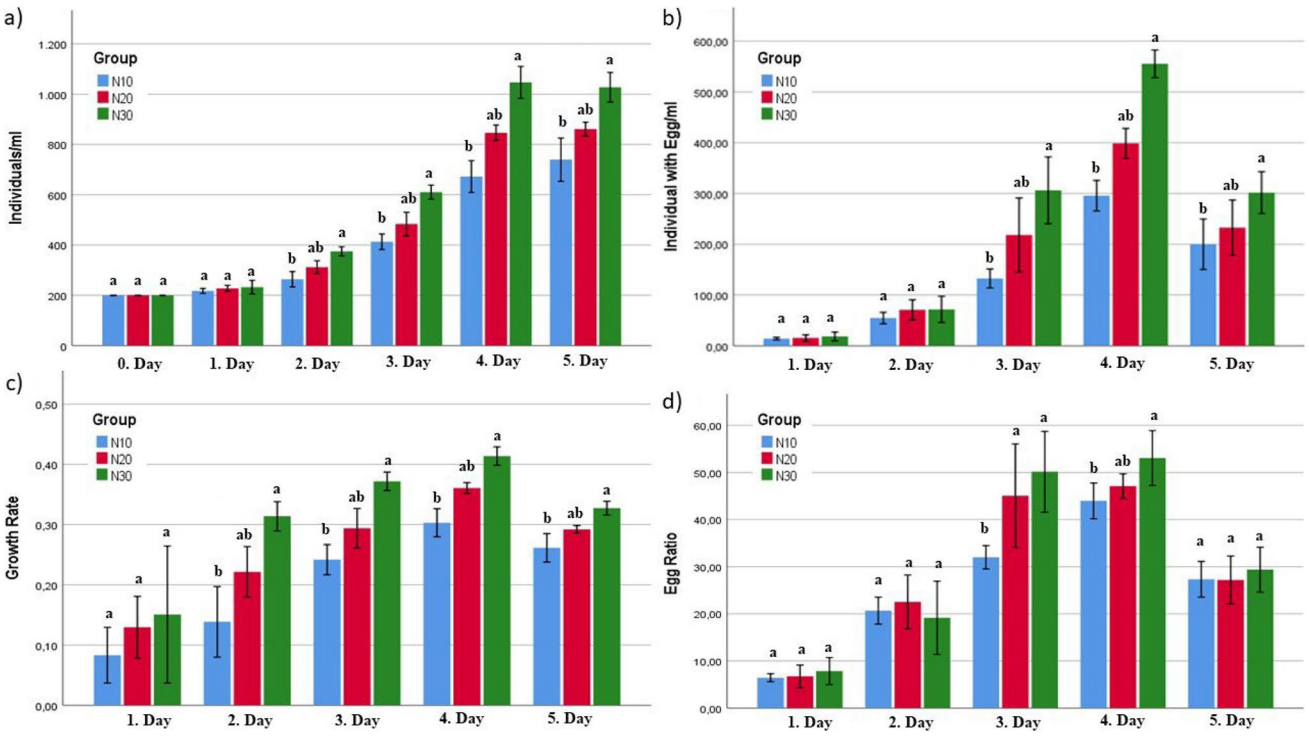


**Fig. 1** Population parameters **a**) individuals $mL^{-1}$, **b**) individual with eggs $mL^{-1}$, **c**) growth rate, **d**) egg ratio] of rotifers fed with different ratios of *Nannochloropsis* sp. Each bar represents the mean of three experiments ($n$=3). Error bars with confidence intervals (95%) are shown

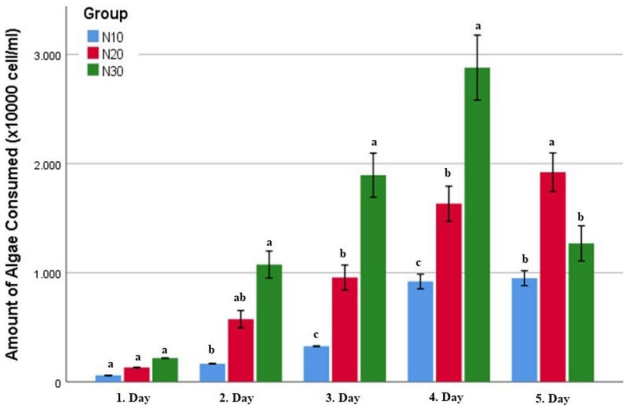


**Fig. 2** The amount of algae consumed by the trial groups during the experimental days. Each bar represents the mean of three experiments ($n$=3). Error bars with confidence intervals (95%) are shown

Among fatty acids, arachidonic acid (C20:4n-6, ARA) and eicosapentaenoic acid (C20:5n-3, EPA) were higher in the N20 group compared to the other groups. Docosapentaenoic acid (C22:5n-3, DPA) was higher in the N10 group compared to the other groups ($P < 0.05$). Docosahexaenoic acid (C22:6n-3, DHA) was not detected in any group. The n-3/n-6 ratio was higher in the N30 group ($P < 0.05$) (Table 2).

## Discussion

*Nannochloropsis* sp. is the preferred microalgae species for *B. plicatilis* production. It is commonly utilized as rotifer feed in larval production (Haas et al. 2016), green water production (Tendencia et al. 2015), as well as in fresh (Patil et al. 2007), spray-dried (Harel et al. 2002), freeze-dried (Tibaldi et al. 2015), and paste forms (Schwarz et al. 2008). *Nannochloropsis* sp. is favored in rotifer culture for larval breeding due to its high nutritional value, appropriate size, and high reproductive ability (Pérez-Legaspi et al. 2018; Eryalçın, 2019).

Results of previous studies have demonstrated that rotifer culture is influenced by both the level and type of feed (Nandini et al. 2007; Abd Rahman et al. 2018). Similarly, the findings of this study indicate a positive correlation between the increasing density of microalgae used in rotifer culture and population growth. In the present study, groups of N10, N20, N30 were employed as feed levels. Among these concentrations, the lowest culture performance was observed in the N10 group. Below this level, it can be deemed a limiting factor for rotifer culture. The best culture performance was seen in the N30 group. In the pre-feeding study conducted by the author before this research, rotifers cultured with a concentration of $40x10^6$ cells $mL^{-1}$ did not give better culture performance than those cultured with a concentration of $30x10^6$ cells $mL^{-1}$.

The foundation of successful marine fish larval culture hinges on the cultivation of rotifers. One crucial factor affecting this success is the type and density of the feed used in rotifer culture (Lubzens 1987; Lubzens et al. 2001; Rahman et al. 2018). Comparing the results of this study with similar research involving *Nannochloropsis* sp., Waqalevu et al. (2019) reported increased growth and egg rate in rotifer cultures. They achieved this by using frozen *Nannochloropsis oculata* and cultivating different rotifer morphotypes (ss-type and L-type). Rahman et al. (2018) showed that the growth rate rose with an increase in microalgae density in rotifers fed with *Nannochloropsis* sp., reaching its peak at a density of $0.3x10^6$ cells $mL^{-1}$. On the other hand, Lee and Tamaru (1993) recommended a density range of $10\text{-}20x10^6$ cells $mL^{-1}$ of *N. oculata* for intensive cultivation in *B. plicatilis* culture. However, in the study conducted by Hoff and Snell (2001), the recommended microalgae density to achieve optimum nutritional values was $0.1x10^6$ cells $mL^{-1}$. Sunil et al. (2024) found that *N. salina* resulted in the highest growth of *B. rotundiformis* when fed at a density of $8x10^6$ cells $mL^{-1}$. When comparing this study with others in the literature, the differing recommended microalgae densities for optimal rotifer culture performance can be attributed to various factors. These include rotifer density, water temperature, light transmittance, water quality, and genetic differences, as noted by Hino and Hirano (1976, 1977) and Lubzens et al. (1980). Additionally, variables such as microalgae culture techniques and nutritional components may also play a role.

Furthermore, in this study, a decrease was observed in the number of individuals, as well as in the number and proportion of individuals with eggs in the N30 group on the 5th day. This decline can be attributed to the reduction in the amount of microalgae available per individual. The microalgae density did not increase in proportion to the rising number of rotifers while maintaining a constant microalgae density. Additionally, it was noted that the accumulation of algal matter at the bottom of the vessels was higher in the group fed with high density compared to the other groups. This might have had a negative impact on the population. Furthermore, this sediment of algal matter likely exerted a negative influence on algal consumption rates. The N30 group, experiencing the most intense feeding pressure on the fifth day, exhibited a greater degree of algal precipitation compared to other groups. This sediment can potentially impact rotifer feeding rates through two mechanisms: firstly, by increasing turbidity (Miquelis et al. 1998), and secondly, by promoting bacterial growth. The latter effect arises from the sediment itself serving as a substrate for bacteria, compounded by the additional bacterial load released from the digestive tracts of deceased rotifers (Haché & Plante 2011). These observations align with previous research that highlights the negative impact of culture performance on algal consumption rates.

**Table 1** Biochemical analysis results of microalgae and *B. plicatilis* fed with different concentrations of *Nannochloropsis* sp. (% dry weight)

| | *Nannochloropsis* sp. | N10 | N20 | N30 |
|---|---|---|---|---|
| Crude Protein | 36.71±0.62 | 52.93±0.81$^{a}$ | 53.59±0.42$^{a}$ | 54.41±0.53$^{a}$ |
| Crude Lipid | 21.4±0.88 | 32.8±0.37$^{b}$ | 33.9±0.33$^{a}$ | 34.5±0.45$^{a}$ |
| Carbohydrate | 25,24±0.77 | 5,88±0.80$^{a}$ | 6.27±0,68$^{a}$ | 4.50±0,74$^{b}$ |
| Ash | 16.65±0.28 | 5.88±0.19$^{a}$ | 6.24±0.23$^{a}$ | 6.59±0.18$^{a}$ |

The difference between the means shown with different letters on the same line is statistically significant ($p<0.05$)

Values are given as ($n$=3;mean±sd)

N10:10x10$^{6}$, N20:20x10$^{6}$ and N30:30x10$^{6}$ cells mL$^{-1}$ of *Nannochloropsis* sp

**Table 2** Fatty acid analysis results of microalgae and rotifers fed with different concentrations of *Nannochloropsis* sp.

| Fatty acids (%) | *Nannochloropsis* sp. | N10 | N20 | N30 |
|---|---|---|---|---|
| 14:0 | - | - | 0.22 ±0.00$^{b}$ | 0.5 ±0.00$^{a}$ |
| 15:0 | 0.28±0.00 | 0.34 ±0.00$^{c}$ | 0.64 ±0.02$^{b}$ | 1.53 ±0.01$^{a}$ |
| 16:0 | 21.8±0.08 | 13.18±0.04$^{b}$ | 15.17±0.05$^{a}$ | 9.63 ±0.03$^{c}$ |
| 17:0 | 0.19±0.00 | 0.45 ±0.01$^{c}$ | 0.63 ±0.01$^{b}$ | 0.72 ±0.01$^{a}$ |
| 18:0 | - | 0.85 ±0.00$^{c}$ | 2.11 ±0.01$^{b}$ | 6.76 ±0.04$^{a}$ |
| 20:0 | - | 0.26 ±0.00$^{b}$ | 0.70 ±0.00$^{a}$ | 0.58 ±0.00$^{ab}$ |
| ΣSFA[1] | **22.27±0.09** | **15.08±0.02$^{b}$** | **19.47±0.02$^{ab}$** | **19.72±0.02$^{a}$** |
| 16:1n-7 | 18.43±0.03 | 6.45±0.04$^{b}$ | 14.01±0.05$^{a}$ | 8.34 ±0.04$^{ab}$ |
| 18:1n-9c(OLA) | 9.11±0.01 | 8.50 ±0.04$^{b}$ | 9.80 ±0.05$^{ab}$ | 13.70 ±0.05$^{a}$ |
| 18:1n-7c | - | 2.13 ±0.02$^{b}$ | 2.58 ±0.02$^{ab}$ | 2.72 ±0.02$^{a}$ |
| 20:1n-9 | - | 1.97 ±0.01$^{b}$ | 2.21 ±0.02$^{ab}$ | 2.34 ±0.02$^{a}$ |
| 22:1n-9 | - | 0.69 ±0.00$^{a}$ | 0.44 ±0.00$^{b}$ | 0.45 ±0.00$^{ab}$ |
| ΣMUFA[2] | **27.54±0.04** | **19.74±0.02$^{b}$** | **29.04±0.03$^{a}$** | **27.55 ±0.03$^{ab}$** |
| 16:2n-4 | - | - | - | - |
| 18:2n-6(LOA) | 5.27±0.01 | 0.50 ±0.00$^{ab}$ | 0.32 ±0.00$^{b}$ | 0.82 ±0.00$^{a}$ |
| 18:3n-6(γLNA) | - | - | - | - |
| 20:3n-6 | - | - | - | - |
| 20:4n-6(ARA) | 2.21±0.01 | 2.37 ±0.02$^{b}$ | 3.20 ±0.02$^{a}$ | 3.03 ±0.02$^{ab}$ |
| Σn-6PUFA[3] | **7.48±0.02** | **2.87±0.01$^{b}$** | **3.52±0.01$^{ab}$** | **3.85±0.01$^{a}$** |
| 18:3n-3(αLNA) | - | - | - | - |
| 20:3n-3 | - | - | - | - |
| 20:5n-3(EPA) | 13.12±0.05 | 8.66±0.04$^{b}$ | 15.98±0.07$^{a}$ | 14.7±0.05$^{a}$ |
| 22:5n-3(DPA) | - | 3.12±0.02$^{a}$ | 2.68±0.02$^{b}$ | 2.94±0.02$^{ab}$ |
| 22:6n-3(DHA) | - | - | - | - |
| Σn-3PUFA[4] | **13.12±0.05** | **11.78±0.03$^{b}$** | **18.66±0.05$^{a}$** | **17.64±0.04$^{ab}$** |
| Total | **70.41±0.19** | **49.47±0.02$^{b}$** | **70.69±0.03$^{a}$** | **68.76±0.02$^{ab}$** |
| n-3/n-6 | **1.75±0.00** | **4.10±0.02$^{b}$** | **5.30±0.03$^{a}$** | **4.58±0.03$^{ab}$** |
| EPA/ARA | **5.94±0.03** | **3.65±0.03$^{b}$** | **4.99±0.05$^{a}$** | **4.85±0.04$^{ab}$** |

The difference between the means shown with different letters on the same row is statistically significant ($p<0.05$). Values are given as (n=3;mean±sd). Values are given as % of area on the GC-MS graph. 1-*ΣSFA*; Total Saturated Fatty Acid, *2-ΣMUFA*; Total Mono Unsaturated Fatty Acids, *3-Σn-6PUFA*; Total n-6 Poly Unsaturated Fatty Acids, *4-Σn-3PUFA*; Total n-3 Poly Unsaturated Fatty Acids. N10:10x10$^{6}$, N20:20x10$^{6}$ and N30:30x10$^{6}$ cells mL$^{-1}$ of *Nannochloropsis* sp

## Proximate composition and fatty acid analysis

Marine fish larvae need to acquire sufficient nutrients and essential fatty acids for their survival and growth (Izquierdo and Koven 2011; Eryalçın, 2019). Zooplankton play a crucial role in transferring these nutrients to the larvae. Although the nutritional values of live foods are of vital importance for aquaculture, they have not yet been fully optimized (Izquierdo et al. 2000; Monroig et al. 2006; Eryalçın, 2018).

The protein content of rotifers varied between 28-67% depending on the feeding protocol and different growth stages (Lubzens et al. 1989; Øie et al. 1997; Srivastava et al. 2006). In this study, protein ratios between (52.93-54.41%) were observed. When the maximum protein ratios of previous studies on similar subject were analysed, Caric et al. (1993), Jeeja et al. (2011) and Yin et al. (2013) found values lower than the protein ratio of this study, while Khoa et al. (2021) and Sunil et al. (2024) found values higher than the protein ratio of this study. The protein ratio in the diet required for larval feeding is expected to be between 30-50% depending on the species, age and feeding habits of the larvae (Caric et al. 1993). All groups in this study met the protein ratio required for larval feeding.

One of the most important features of using of *Nannochloropsis* sp. in aquaculture production is its richness in EPA fatty acids. EPA is an omega-3 fatty acid known for stabilizing blood circulation, aiding in the formation of the heart and blood vessels, and playing a crucial role in the development of brain cells, bones, and eye organs, as well as in protecting heart cells and vessels. It also contributes to the growth and development of nerve cells. ARA has a positive effect on stress resistance, pigmentation, growth, and survival rate and is involved in the formation of eicosanoids for marine fish larvae. The absence of these fatty acids can lead to dysfunctions in the works and functions of the nervous system during the developmental process (Wiradana et al. 2020). DHA belongs to the omega-3 family and plays a significant role in transmitting nerve impulses to the brain and initiating nerve commands. It also contributes to blood production and aids in heart and vascular movements (Gammone et al. 2018). This study showed that essential fatty acids EPA and ARA were effectively incorporated by the rotifer with an increase in microalgae density fed. As *Nannochloropsis* sp. lacks DHA, larvae must be enriched with this fatty acid before feeding. When similar studies were examined, Eryalçın (2019) found that ARA and EPA were higher in the rotifer group fed with freshly cultured *N. oculata* compared to the rotifer group fed with spray-dried *N. oculata*. Waqalevu et al. (2019) reported that fatty acid composition varied according to different morphotypes. Additionally, researchers have noted that factors such as preferred environmental conditions in rotifer culture (Begum et al. 2021), microalgae harvesting techniques (Eryalçın, 2019), the utilization of different microalgae and probiotic bacteria (Jeeja et al. 2011), the diversity of culture systems, and the renewal rates of the culture medium (Kobayashi et al. 2008) can influence the biochemical content and fatty acid profile of rotifer. Moreover, one reason for the difference in fatty acid profiles may be attributed to the fatty acid conversion metabolism of zooplankton, a process known as De nova (Nanton and Castell 1999).

Proteins and lipids are the main players in animal nutrition. Carbohydrates are less important, but they serve as energy storage for living things (Whyte & Nagata 1990; Caric et al 1993). Growth and development of the organism may be adversely affected if the metabolism provides energy from proteins and lipids in the absence of carbohydrates. To prevent this, the organism's diet should contain sufficient carbohydrate to support growth and development. *Nannochloropsis* sp. can provide enough carbohydrates for rotifers (Caric et al 1993; Chakraborty et al 2007; Hannah et al 2013; Sunil et al 2024).

## Conclusıon

In conclusion, successful rotifer culture can be achieved with *Nannochloropsis* sp. The nutrient content of the microalgae is of paramount importance in producing rotifer biomass. The present study noted that feeding rotifers with *Nannochloropsis* sp. at a density of $30x10^6$ cells $mL^{-1}$ (N30) led to successful results in increasing the rotifer population. This demonstrates that an increase in concentration of *Nannochloropsis* sp. has a positive effect on population growth.

**Author contributions** All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Mustafa İbrahim Osmanoğlu. The first draft of the manuscript was written by Mustafa İbrahim Osmanoğlu. Mahmut Elp and Yaşar Durmaz commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**Funding** Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK). No funding was received to assist with the preparation of this manuscript

**Data availability** Data can be obtained from the corresponding author upon reasonable request.

## Declarations

**Competing interests** The authors declare no competing interests.

## References


Abd Rahman AR, Cob ZC, Jamari Z, Mohamed AM, Toda T, Ross OH (2018) The effects of microalgae as live food for *Brachionus plicatilis* (Rotifer) in intensive culture system. Trop Life Sci Res 29:127–138

Agh N, Sorgeloos P (2005) Handbook of protocols and guidelines for culture and enrichment of live food for use in larviculture. Aquatic Animals Research Center, Urmia University, Urmia, p 60

Ahmad AL, Yasin NM, Derek CJC, Lim JK (2014) Comparison of harvesting methods for microalgae *Chlorella* sp. and its potential use as a biodiesel feedstock. Environ Technol 35:2244–2253

AOAC (2010) Official Methods of Analysis, 18th Edn, revision 3. AOAC, Washington

Begum N, Siddiky MM, Ahmmed S (2021) Comparison of growth performance of live feed microalgae and rotifer (*Brachionus* sp.) under different feeding medium in outdoor culture condition. World J Biol Pharm Health Sci 5:025–032

Caric M, Sanko-Njire J, Skaramuca B (1993) Dietary effects of different feeds on the biochemical composition of the rotifer (*Brachionus plicatilis* Muller). Aquaculture 110:141–150

Chakraborty RD, Chakraborty K, Radhakrishnan EV (2007) Variation in fatty acid composition of *Artemia salina* nauplii enriched with microalgae and baker's yeast for use in larviculture. J Agric Food Chem 55:4043–4051

Conceição LE, Yúfera M, Makridis P, Morais S, Dinis MT (2010) Live feeds for early stages of fish rearing. Aquac Res 41:613–640

Coutinho P, Ferreira M, Freire I, Otero A (2020) Enriching rotifers with "Premium" microalgae: *Rhodomonas lens*. Mar Biotechnol 22:118–129

Das P, Mandal SC, Bhagabati SK, Akhtar MS, Singh SK (2012) Important live food organisms and their role in aquaculture. Front Aquacult 5:69–86

Edmondson WT (1960) Reproductive rates of rotifers in natural populations. Memorie dell'Istituto Italiano di Idrobiologia Dott. Marco de Marchi 12:21–77

Eryalçın KM (2018) Effects of different commercial feeds and enrichments on biochemical composition and fatty acid profile of rotifer (*Brachionus plicatilis*, Muller 1786) and *Artemia franciscana*. Turk J Fish Aquat Sci 18:81–90

Eryalçın KM (2019) Nutritional value and production performance of the rotifer *Brachionus plicatilis* Müller, 1786 cultured with different feeds at commercial scale. Aquaculture Int 27:875–890

Folch JL, Lees M, Stanley GHS (1957) A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226:497–509

Gammone MA, Riccioni G, Parrinello G, D'Orazio N (2018) Omega-3 polyunsaturated fatty acids: Benefits and endpoints in sport. Nutrients. 11:46

Grubišić MR, Dulić ZP, Stanković MB, Živić IM, Bjelanović KZ, Spasić MM, Marković ZZ (2012) Importance of zooplankton as live feed for carp larvae. In:Proceedings of 6th Central European Congress on Food-CEFood Congress. Institute of Food Technology, Novi Sad (Serbia). https://hdl.handle.net/21.15107/rcub_agrospace_2778. Accessed 08.03.2023

Guillard RR, Ryther JH (1962) Studies of marine planktonic diatoms: I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. Can J Microbiol 8:229–239

Guiry MD, Guiry GM (2020) AlgaeBase. Worldwide electronic publication. National University of Ireland, Galway https://www.algaebase.org. Accessed 08.03.2023

Haas S, Bauer JL, Adakli A, Meyer S, Lippemeier S, Schwarz K, Schulz C (2016) Marine microalgae *Pavlova viridis* and *Nannochloropsis* sp. as n-3 PUFA source in diets for juvenile European sea bass (*Dicentrarchus labrax* L.). J Appl Phycol 28:1011–1021

Haché R, Plante S (2011) The relationship between enrichment, fatty acid profiles and bacterial load in cultured rotifers (*Brachionus plicatilis* L-strain) and *Artemia* (*Artemia salina* strain Franciscana). Aquaculture 311:201–208

Hamre K, Yúfera M, Rønnestad I, Boglione C, Conceição LE, Izquierdo M (2013) Fish larval nutrition and feed formulation: knowledge gaps and bottlenecks for advances in larval rearing. Rev Aquac 5:26–58

Hannah C, Mani M, Ramasamy R (2013) Evaluation of the biochemical composition of four marine algae and its nutritional value for brine shrimp. IOSR J Pharm Biol Sci 6:47–51

Harel M, Koven W, Lein I, Bar Y, Behrens P, Stubblefield J, Zohar Y, Place AR (2002) Advanced DHA, EPA and ArA enrichment materials for marine aquaculture using single cell heterotrophs. Aquaculture 213:347–362

Hawkyard M, Stuart K, Langdon C, Drawbridge M (2016) The enrichment of rotifers (*Brachionus plicatilis*) and *Artemia franciscana* with taurine liposomes and their subsequent effects on the larval development of California yellowtail (*Seriola lalandi*). Aquac Nutr 22:911–922

Hino A, Hirano R (1976) Ecological studies of bisexual reproduction in the rotifer *Brachionus plicatilis*. I. General aspects of bisexual reproduction inducing factors. Bull Jap Sci Fish 42:1093–1099

Hino A, Hirano R (1977) Ecological studies on the mechanism of bisexual reproduction in the rotifer *Brachionus plicatilis*. II. Effects of cumulative parthenogenetic generation on the frequency of bisexual reproduction. Bull Jap Soc Sci Fish 43:1147–1155

Hoff FH, Snell TW (2001) Microalgae culture. Plankton Culture Manual, 5th edn. Florida Aqua Farms Inc., Dade City, pp 27–59

Izquierdo MS, Socorro J, Arantzamendi L, Hernández-Cruz CM (2000) Recent advances in lipid nutrition in fish larvae. Fish Physiol Biochem 22:97

IzquierdoKoven MS (2011) Lipids. In: Holt J (ed) Larval fish nutrition. Wiley-Blackwell, Oxford, pp 47–82

James CM, Rezeq TA (1988) Effects of different cell densities of a *Chlorella capsulata* and a marine *Chlorella* sp. for feeding the rotifer *Brachionus plicatilis*. Aquaculture 69:43–56

Jeeja PK, Imelda J, Paulraj R (2011) Nutritional composition of rotifer (*Brachionus plicatilis* Muller) cultured using selected natural diets. Indian J Fish 58:59–65

Jeffs A, O'Rorke R (2020) Feeding and nutrition of crustacean larvae. The natural history of the Crustacea: Developmental biology and larval ecology. 7:309

Kates M (1986) Lipid extraction procedures. Techniques of lipidology: isolation, analysis and identification of lipids. Elsevier, Amsterdam, pp 106–107

Khoa TND, Waqalevu V, Honda A, Matsui H, Truong NX, Sakaguchi K, Kawaii H, Ishikawa M, Shiozaki K, Kotani T (2021). Enrichment effects of fermented by-product of Shochu distillery on *Brachionus plicatilis* sp. rotifer and larviculture performance in Japanese flounder (*Paralichthys olivaceus*). Aquaculture 535:736352

Kobayashi T, Nagase T, Hino A, Takeuchi T (2008) Effect of combination feeding of *Nannochloropsis* and freshwater *Chlorella* on the fatty acid composition of rotifer *Brachionus plicatilis* in a continuous culture. Fish Sci 74:649–656

Lee CS, Tamaru CS (1993) Live larval food production at Oceanic Institute, Hawaii. In: McPey JP (ed) CRC Handbook of Mariculture: Crustacean Aquaculture, 2nd edn. I. CRC Press, Boca Raton, pp 15–28

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Lubzens E (1987) Raising rotifers for use in aquaculture. Hydrobiologia 147:245–255

Lubzens E, Fishler R, Berdugo-White V (1980) Induction of sexual reproduction and resting egg production in *Brachionus plicatilis* reared in sea water. Hydrobiologia 73:55–58

Lubzens E, Tandler A, Minkoff G (1989) Rotifers as food in aquaculture. Hydrobiologia 186:387–400

Lubzens E, Zmora O, Barr Y (2001) Biotechnology and aquaculture of rotifers. Hydrobiologia 446/447:337–353

Martinez-Silva MA, Audet C, Winkler G, Tremblay R (2018) Prey quality impact on the feeding behavior and lipid composition of winter flounder (*Pseudopleuronectes americanus*) larvae. Aquacult Fish 3:145–155

Miquelis A, Rougier C, Pourriot R (1998) Impact of turbulence and turbidity on the grazing rate of the rotifer *Brachionus calyciflorus* (Pallas). Hydrobiologia 386:203–211

Monroig Ó, Navarro JC, Amat F, González P, Bermejo A, Hontoria F (2006) Enrichment of *Artemia* nauplii in essential fatty acids with different types of liposomes and their use in the rearing of gilthead sea bream (*Sparus aurata*) larvae. Aquaculture 251:491–508

Nandini S, Sarma SSS, Amador-López RJ, Bolaños-Muñioz S (2007) Population growth and body size in five rotifer species in response to variable food concentration. J Freshw Ecol 22:1–10

Nanton DA, Castell JD (1999) The effects of temperature and dietary fatty acids on the fatty acid composition of harpacticoid copepods, for use as a live food for marine fish larvae. Aquaculture 175:167–181

Øie G, Makridis P, Reitan KI, Olsen Y (1997) Protein and carbon utilization of rotifers (*Brachionus plicatilis*) in first feeding of turbot larvae (*Scophthalmus maximus* L.). Aquaculture 153:103–122

Özçiçek E, Can E, Yılmaz K, Seyhaneyıldız Can Ş (2017) Usage of microalgae as a sustainable food source in aquaculture. Ege J Fish Aquat Sci 34:347–354

Patil V, Källqvist T, Olsen E, Vogt G, Gislerød HR (2007) Fatty acid composition of 12 microalgae for possible use in aquaculture feed. Aquac Int 15:1–9

Pérez-Legaspi IA, Guzmán-Fermán BM, Moha-León JD, Ortega-Clemente LA, Valadez-Rocha V (2018) Effects of the biochemical composition of three microalgae on the life history of the rotifer *Brachionus plicatilis* (Alvarado strain): an assessment. Int J Limnol 54:20

Rahman AR, Cob ZC, Jamari Z, Mohamed AM, Toda T, Ross OH (2018) The effects of microalgae as live food for *Brachionus plicatilis* (rotifer) in intensive culture system. Trop Life Sci Res 29:127

Rainuzzo JR, Reitan KI, Olsen Y (1997) The significance of lipids at early stages of marine fish: a review. Aquaculture 155:103–115

Ramlee A, Chembaruthy M, Gunaseelan H, Yatim SRM, Taufek H, Rasdi NW (2021) Enhancement of nutritional value on zooplankton by alteration of algal media composition: a review. IOP Conf Ser: Earth Environ Sci 869:012006

Rasdi NW, Ikhwannuddin M, Azani N, Ramlee A, Yuslan A, Suhaimi H, Ashaari U, Arshad A (2020) The effect of different feeds on the growth, survival and reproduction of rotifer, *Brachionus plicatilis*. J Env Biol 41:1275–1280

Reitan KI, Rainuzzo JR, Øie G, Olsen Y (1997) A review of the nutritional effects of algae in marine fish larvae. Aquaculture 155:207–221

Sales R, Lopes RG, Derner RB, Tsuzuki MY (2022) Concentrated microalgal biomass as a substitute for fresh microalgae produced on site at hatcheries. Aquacult Res 53:5771–5786

Schwarz MH, Craig SR, Delbos BC, McLean E (2008) Efficacy of concentrated algal paste during greenwater phase of cobia larviculture. J Appl Aquacult 20:285–294

Srivastava A, Hamre K, Stoss J, Chakrabarti R, Tonheim SK (2006) Protein content and amino acid composition of the live feed rotifer (*Brachionus plicatilis*): With emphasis on the water soluble fraction. Aquaculture 254:534–543

Suchar VA, Chigbu P (2006) The effects of algae species and densities on the population growth of the marine rotifer, *Colurella dicentra*. J Exp Mar Biol Ecol 337:96–102

Sunil SC, Krishnan A, Jacob V, Varghese SP, Chaithanya ER (2024) Optimizing the dietary concentration of marine microalgae *Nannochloropsis salina* and *Chaetoceros calcitrans* for the enhancement of productivity and nutritional composition of the rotifer *Brachionus rotundiformis* (Tschugunoff, 1921). J Appl Phycol. https://doi.org/10.1007/s10811-024-03254-6

Tendencia EA, Bosma RH, Verdegem MC, Verreth JA (2015) The potential effect of greenwater technology on water quality in the pond culture of *Penaeus monodon Fabricius*. Aquac Res 46:1–13

Theilacker GH, McMaster MF (1971) Mass culture of the rotifer *Brachionus plicatilis* and its evaluation as a food for larval anchovies. Mar Biol 10:183–188

Tibaldi E, Zittelli GC, Parisi G, Bruno M, Giorgi G, Tulli F, Venturini S, Tredici MR, Poli BM (2015) Growth performance and quality traits of European sea bass (*D. labrax*) fed diets including increasing levels of freeze-dried *Isochrysis* sp. (T-ISO) biomass as a source of protein and n-3 long chain PUFA in partial substitution of fish derivatives. Aquaculture 440:60–68

Vidhya K, Uthayakumar V, Muthukumar S, Munirasu S, Ramasubramanian V (2014) The effects of mixed algal diets on population growth, egg productivity and nutritional profiles in. J Basic Appl Zool 67:58–65

Waqalevu V, Matsui H, Honda A, Dossou S, Yamamoto A, Shiozaki K, Kotani T (2019) Enrichment effect on two *Brachionus plicatilis* sp. complex morphotypes fed three nutritional enrichment diets. Aquacult Sci 67:139–155

Whyte JNC, Nagata WD (1990) Carbohydrate and fatty acid composition of the rotifer *Brachionus plicatilis* fed monospecific diets of yeast or phytoplankton. Aquaculture 89:263–272

Wiradana PA, Sani MD, Susilo RJK, Ansori ANM, Septiani NNS, Yusup DS, Soegianto A (2020) Population-level of *Nannochloropsis* sp. as an enrichment diet for marine rotifer *Brachionus rotundiformis* in mass culture tanks. Ecol Environ Conservat 26:S12–S17

Woods C, Woolley L, Partridge G, Chen M, Haney EF, Hancock RE, Buller N, Currie A (2022) Assessing the activity of antimicrobial peptides against common marine bacteria located in rotifer (*Brachionus plicatilis*) cultures. Probiot Antimicrob Proteins 14:620–629

Yin XW, Min WW, Lin HJ, Chen W (2013) Population dynamics, protein content, and lipid composition of *Brachionus plicatilis* fed artificial macroalgal detritus and *Nannochloropsis* sp. diets. Aquaculture 380:62–69

Yoshimatsu T, Hossain MA (2014) Recent advances in the high-density rotifer culture in Japan. Aquac Int 22(5):1587–1603