



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

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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: 10×10^6 , N20: 20×10^6 , N30: 30×10^6 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 30×10^6 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

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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 zooplankton 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*).

Materials 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 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) 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: 10×10^6 ; N20: 20×10^6 ; N30: 30×10^6 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 continuously aerated with a ring system. Resun air 8000 (Air flow 540 L h⁻¹, pressure $\geq$ 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 amictic females (Edmondson 1960; Theilacker and McMaster 1971; James and Rezeq 1988).

$$\text{Population density} : \frac{\text{Number of rotifers}}{\text{mL}} \quad (1)$$

$$\text{Instantaneous Growth rate} : r = \frac{\ln N_t - \ln N_o}{t} \quad (2)$$

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

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).

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

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 boron trifluoride 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 10×10^6 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 30×10^6 – 40×10^6 cells mL^{-1} . In those fed at a concentration above 45×10^6 cells mL^{-1} , the lifespan of the rotifers did not exceed 3 days. According to these results, three different *Nannochloropsis* sp. concentrations (N10: 10×10^6 ; N20: 20×10^6 ; N30: 30×10^6 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 $53 \pm 1.9\%$ 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 $2,880 \pm 98 \times 10^4$ 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).

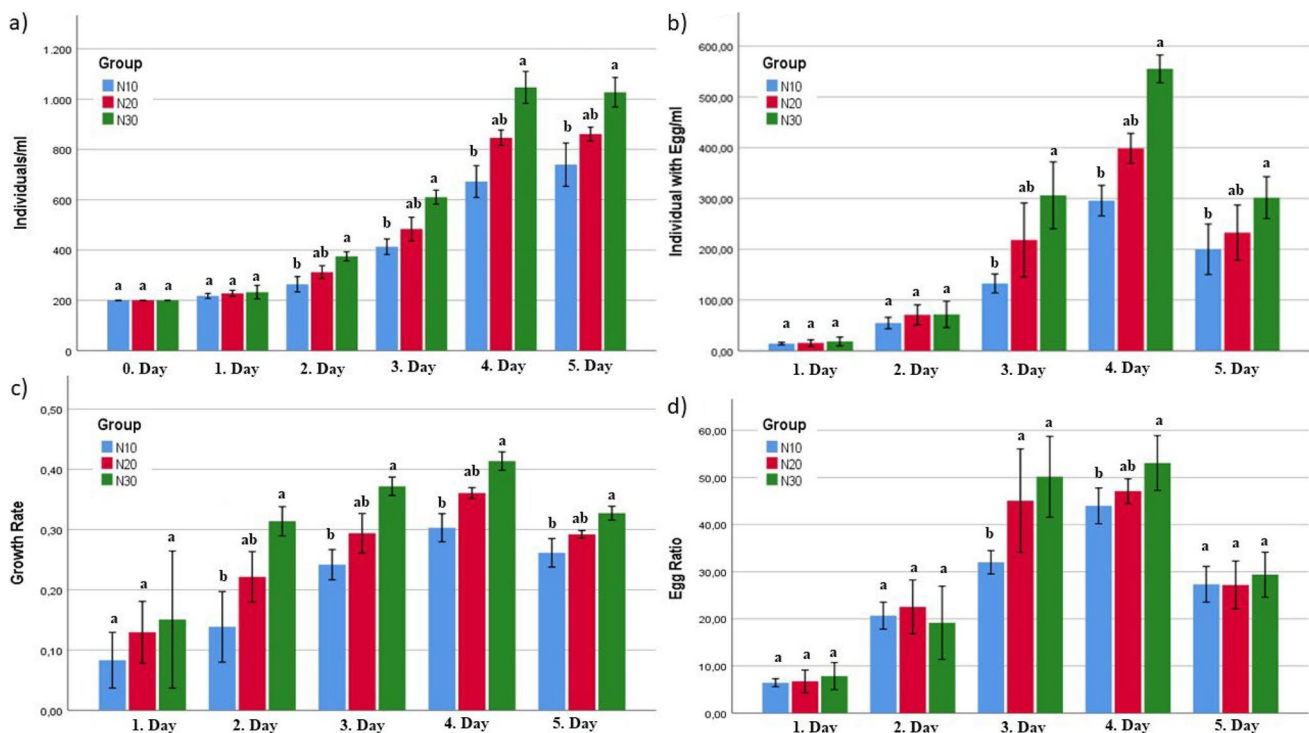


Fig. 1 Population parameters **a)** individuals mL^{-1} , **b)** individual with eggs mL^{-1} , **c)** growth rate, **d)** egg ratio] of rotifers fed with different ratios of *Nannochloropsis* sp. Each bar represents the mean of three experiments ($n=3$). Error bars with confidence intervals (95%) are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

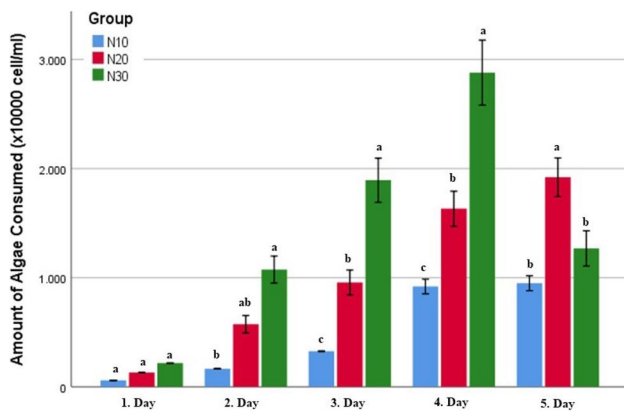


Fig. 2 The amount of algae consumed by the trial groups during the experimental days. Each bar represents the mean of three experiments ($n=3$). Error bars with confidence intervals (95%) are shown

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 40×10^6 cells mL^{-1} did not give better culture performance than those cultured with a concentration of 30×10^6 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 0.3×10^6 cells mL^{-1} . On the other hand, Lee and Tamaru (1993) recommended a density range of $10\text{--}20 \times 10^6$ 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 0.1×10^6 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 8×10^6 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 (Miquelís 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)

	<i>Nannochloropsis</i> 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)

N10:10x10⁶, N20:20x10⁶ and N30:30x10⁶ 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 (%)	<i>Nannochloropsis</i> 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. N10:10x10⁶, N20:20x10⁶ and N30:30x10⁶ 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).

Conclusion

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 30×10^6 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.

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

Declarations

Competing interests The authors declare no competing interests.

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