

Production of Rotifer, *Brachionus Plicatilis* (MÜLLER 1786) in a Continuous Algae System in a Helical Photobioreactor

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Abstract

In the aquaculture sector, live feeds are of great importance, especially in the larval stages of some economic sea fish such as sea bream, sea bass, turbot and coral. Rotifer is an excellent food source for many marine fish larvae. Today, productivity in marine fish larvae production in hatcheries is largely dependent on *Brachionus plicatilis*. Rotifers are an ideal food for larvae during the first feeding period when the mouth opening is small. Rotifer is an excellent food source for many marine fish larvae. Today, productivity in marine fish larvae production in hatcheries is largely dependent on *B. plicatilis*. Rotifers are an ideal food for larvae during the first feeding period when the mouth opening is small. The most commonly used rotifer species in marine fish farming is *B. plicatilis*. The use of microalgae in the feeding of rotifers provides a more effective and reliable enrichment. In addition, microalgae have positive effects on water quality. Microalgae transfer their rich fatty acid content to the larva through the rotifer, improving the survival rate and growth of the larva. *Nannochloropsis* sp. (*Eustigmatophyceae*) are grown in marine fish hatcheries as bait for rotifers and to create a 'green water effect' in larval tanks. Continuous (compared to batches) microalgae production requires the adoption of specially designed fully enclosed and controlled photobioreactors, for example: flat sheet systems, tubular systems and coil type systems. Tubular systems are the most widely used commercial systems. Coil type systems were developed mainly to improve space utilization compared to other categories. It is among its most important advantages. In the helical photobioreactor designed in the study, the *Nannochloropsis oculata* algae species were put into production in a continuous system and using artificial light source (daylight) at a salt concentration of 30‰. Algae intrusion from the helical photobioreactor into the rotifer tank was done continuously for two weeks and the rotifer was harvested from the rotifer tank within 24 hours. The study was repeated three times. Cell increases and specific growth rates of *Rotifer B. plicatilis* were investigated by growing with a continuous algae system. As a result, in the study; Algae produced at maximum density in the continuous system in the helical photobioreactor were given to the *B. plicatilis* tank and harvested daily from the rotifer (*B. plicatilis*) culture tank for 24 hours. During the experiment, the growth performance of *B. plicatilis* increased 10 times on average. It is thought that the results of this study will be evaluated in order to increase the production of rotifers in marine fish farms where larval production is made and an increase in product productivity will be achieved while ensuring continuity in rotifer production.

Keywords: *Nannochloropsis oculata*, *Brachionus plicatilis*, Helical Photobioreactor, Continuous Production System, Growth rate, Cell growth, Salinity

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1. Introduction

Aquaculture is becoming crucial as a potential source for sustainable animal protein production worldwide. Especially fish production is an important sector since it is declining due to natural fishing (FAO 2016). One of the most critical challenges hindering the development and growth of the aquaculture industry is that fish feeds consume at least 60% of total farm income and provide cheap feed (FAO 2016). Therefore, researchers need to try to solve these problems by using live food (phytoplankton and zooplankton) and reducing dependence on artificial feeds (Yousef & Hegab 2017).

In the aquaculture sector, live feeds are of great importance, especially during the larval stages of some economical marine fish such as sea bream, sea bass, turbot and coral (Hoff & Snell 1987, Lubzens *et al.* 1989). Another major challenge is the formulation of suitable feeds for small larval fish whose functional development of the digestive tract is not yet complete (Pedersen & Hjelme land 1988).

Zooplankton are recognized worldwide as a source of animal protein. They play an important role in the aquatic ecosystem by occupying a transitional position in the food chain (Xie, Xiao, Tang, Lu & Cai 2008). It is also very important as a nutrient for many fish (Grubišić *et al.* 2012).

The most commonly used zooplankton species as live bait in marine fish larvae breeding are rotifer and artemia. In order for these two filter-feeding organisms to be a nutritious food for the larvae, they must be enriched with appropriate nutrients. The nutritional content of these organisms varies depending on the type of food used and its concentration in the environment (James *et al.* 1987).

Rotifer is considered an important bait for fish larvae in aquaculture. They help the development of the digestive system of fish larvae (Demir & Diken 2011). In marine fish farming, the quality of the first live bait used immediately after the larvae open their mouths plays an important role in the high survival and growth rates. *Rotifer (Brachionus plicatilis)* is the first live food used in the larval period feeding of marine fish, and it is a live food that has no alternative in the larval period in terms of both size and mobility and is mandatory to be produced in hatcheries. Apart from their size suitable for the mouth opening of the larva, rotifers are a suitable food for the delivery of some nutrients such as essential fatty acids, amino acids, vitamins and minerals to the larvae (Nanton & Castell, 1999; Drillet *et al.* 2011). Rotifer has a complex life cycle due to its high growth rate, sufficient size, high nutrient content and being able to be fed with various types of feed (Jeeja, Joseph & Raj 2011; Kostopoulou, Carmona & Divanach 2012).

The feeds and feeding method used in rotifer cultivation affect the cellular growth and nutritional quality of rotifers. Especially in commercial live feed units where semi-continuous production method is applied, the main purpose of rotifer culture is to have high rotifer density. Commonly used in rotifer nutrition are commercial rotifer foods, powdered microalgae products and live-fresh microalgae produced in the enterprise. Among the rotifer foods, baker's yeast has been reported to provide higher production compared to microalgae based feeds (Qie *et al.* 1994). The nutritional values of cultured rotifers are directly proportional to the protein and fat contents of the foods used. Therefore, the nutrients and feeding methods used are important not only for the production of rotifers in high numbers, but also for the nutritional aspect and the number of harvests (Dhert *et al.* 2001; Dhert *et al.* 2014).

The rotifers to be used in larval feeding should be rich in essential fatty acids (EFA) (George *et al.* 1989). Generally, *Chlorella* sp., *Isochrysis* sp. and *Nannochloropsis* sp, rich in some fatty acids, minerals and vitamins (Chen & Long 1991, Arnold & Holt 1991) are used. In addition, the presence of microalgae in tanks in fish larvae breeding makes an indirect immunological stimulus, reduces the nitrogen and phosphorus load, limits the growth of bacteria and improves water quality (Moretti *et al.* 1999). *N. oculata* is one of the leading microalgae species used as food in many hatcheries where marine fish are grown, especially in rotifer feeding, green water techniques and larval stages of some crustacean species (Duerr *et al.* 1998, Liao *et al.* 2001).

Live feed units where plankton culture is carried out in enterprises where fish are grown are an integral part of cross hatchery (Timur 1992). The most commonly used rotifer species in marine fish farming is *Brachionus plicatilis*. Today, *B. plicatilis* has become one of the indispensable live foods in aquaculture. High efficiency in rotifer production depends on the applied production methods, culture conditions (temperature, salinity, light, etc.) and nutrition regime (Lubzens *et al.* 1989). The type and amount of food used for rotifers are among the factors affecting population growth. Various algae species such as *Chlorella* sp., *Tetraselmis suecica*, *Tetraselmis tetrahele*, *Dunaliella tertiolecta*, *Nannochloropsis oculata*, *Nannochloris* sp., *Isochrysis galbana*, *Phaedactylum tricorutum* are used in rotifer feeding (Chen & Long 1991). Rotifers are tolerant of a wide range of salinity (1-60‰) (Hoff & Snell, 1989). It is stated that the optimum salinity ratio with high population growth in rotifer production is 15-20‰ for S-type rotifers and 30‰ for L-type rotifers (Hagiwara & Hino 1990; Chen 1991). The salinity of the culture medium is reported to be the most important factor preventing the formation of mictic teeth of rotifers, as well as affecting oxygen consumption and swimming movements, as well as having an effect on lorica length and nutrient filtration rate (Qie & Olsen 1993; Fielder *et al.* 2000; Niksa *et al.* 2000). Rotifers can consume nutrients consisting of different sizes of algae, yeast, bacteria and synthetic feeds (Hino & Hirano 1984). The use of microalgae in the feeding of rotifers provides a more effective and reliable enrichment. In addition, microalgae have positive effects on water quality (Hoff & Snell 1989). Microalgae transfer their rich fatty acid content to the larva through the rotifer, improving the survival rate and growth of the larva.

In this trial study, the green microalgae species *Nannochloropsis oculata* (Droop), which is widely used in rotifer feeding in our country, was used. *N. oculata* microalgae was produced by continuous production technique in a helical photobioreactor at 30‰ salt concentration and cell growth and growth rate of rotifer *B. plicatilis* fed with this algae were investigated. The fact that *Nannochloropsis* sp. is within the food size limits that the rotifer can consume will cause both more consumption of these nutrients and a better cell growth and growth rate; It is aimed to investigate this algae-fed rotifers. It is thought that the results of the research will be evaluated in order to increase the production of *B. plicatilis*, the most commonly used *Rotifer* species in marine fish farming, in enterprises where marine fish larvae are raised. In addition, it is thought that it will provide continuity to *Rotifer* production and increase product efficiency.

2. Material and Method

The research was carried out in the Plankton laboratory of Mersin University Faculty of Fisheries. *N. oculata* and *Rotifer B. plicatilis*, which were used as trial material, were obtained from Ç.Ü. obtained from the Faculty of Fisheries. Conway nutrient medium (Walne 1966) was used as an algae nutrient medium. A continuous system (Brown *et al.* 1993) was used in a spiral photobioreactor for microalgae production. Sea water used in the culture of *Nannochloropsis oculata* and *Brachionus plicatilis* was first passed through a 25 µm cartridge filter and then a

UV filter.

Rotifer B. plicatilis was put into production for two weeks at a salt concentration of 30‰ in a continuous algae system in a helical photobioreactor. *B. plicatilis*, which was fed with algae produced in the continuous system in the helical photobioreactor, was put into production by repeating three times. Thus, daily cell increase and growth rates of *B. plicatilis* were determined. *N. oculata* produced in the helical photobioreactor was given to the algae rotifer tanks and harvested from the rotifer tank within 24 hours. During the experiment, daily cell counts and growth rates of *B. plicatilis* were determined.

2.1. Counting method and Calculation of Instant Growth Rate of *Brachionus plicatilis*.

The algae, produced at maximum daily density by continuous production technique in the spiral photobioreactor, were given to the *B. plicatilis* tank and harvested from the rotifer culture bowl in 24-hour periods. Sedgwick Rafter counting camera (Edmondson & Winberg 1971) was used to determine the individual number of *B. plicatilis*. The daily individual increase of *B. plicatilis* was determined. The algae obtained at the specific growth rate were added dropwise to the rotiferin stock culture dish at a dilution (dilution) rate of 7min/mL. Thus, the maximum density of the rotifer was stabilized by continuously entering the algae that reached the maximum density from the stock algae culture container to the rotifer stock container. The rotifer was harvested routinely every day. The culture of *B. plicatilis* has been studied from different perspectives by many researchers. However, its success in mass culture still has the potential to evolve (Kostopoulou, Miliou & Verriopoulos 2015). Best growth rate of *B. plicatilis* Rico-Martinez & Dodson (1992); It was calculated by Hotos (2003) with the following equation.

$$K = \mu = (\ln N_t - \ln N_0) / t$$

K: growth rate, **t:** time, **No:** number of initial rotifer individuals (1)

2.2. Bioreactor Designed in Experiment

In the experiment, a spiral (helical) photobioreactor, which is one of the closed systems, was used (Figure 1). A peristaltic pump was used to circulate the algae in the helical bioreactor. In the system, an artificial (daylight) light source was used indoors.



Figure 1. Helical Photobioreactor designed in experiment

In the spiral system, it was deemed appropriate to add a container in which algae can be collected to the system. Algae was introduced into the rotifer culture vessel with the same flow rate from the harvest vessel where the algae were collected. Daily rotifer harvest was done with the same flow rate. For ideal growth of *N. oculata* algae, temperatures are 24.4-28.0; The pH was tried to be kept in balance between 7.32–9.91. In order to balance the pH in the system, carbon dioxide was added to the environment at regular intervals (1‰). Helical (helozoic) photobioreactor used in the experiment; designed for continuous production under laboratory conditions. By ensuring the continuity of the algae taken into production in the continuous system at maximum density; at the same time, continuous production is aimed at which the rotifer density is fixed at the maximum rate.

3. Results

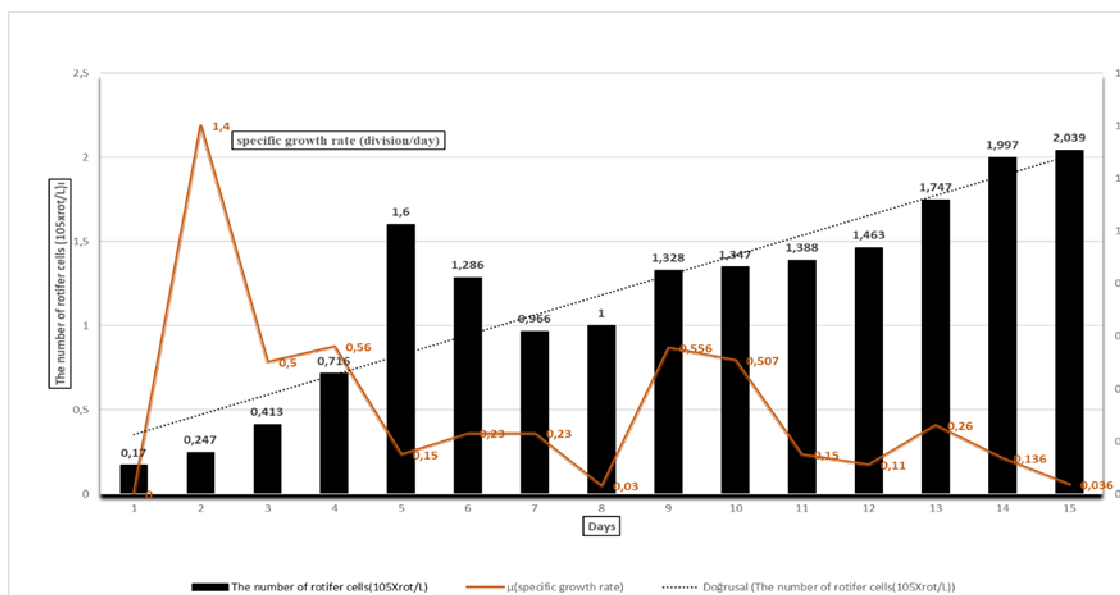
The average initial sowing density of *Brachionus plicatilis* at 30‰ salinity concentration is $1.615 \times 10^5 \pm 175.54$ rotifer/L. *B. plicatilis* rotifer density 1–4. increased logarithmically between days. On the 5th

day, the mean rotifer count was $1,600 \times 10^5$ rotifer/L. Average growth rates were determined as 0.149 divisions/day on the 5th day. The mean number of rotifers on the 6th and 7th days were $1,286 \times 10^5$, $0,966 \times 10^5$ rotifer/L, 6th-7th, respectively. The average growth rate of the days is 0.230-0.230 divisions/day. The mean number of rotifers was $1,328 \times 10^5$ - $1,347 \times 10^5$ rotifer/L on the 9th and 10th days. The mean cell increase in the 11th and 15th days, respectively; $1,388 \times 10^5$ rotifer/L, $1,463 \times 10^5$ rotifer/L, $1,997 \times 10^5$ rotifer/L, $2,039 \times 10^5$ rotifer/L, $2,040 \times 10^5$ rotifer/L. Growth rates were determined as 0.152 divisions/day, 0.111 divisions/day, 0.256 divisions/day, 0.136 divisions/day, 0.036 divisions/day.

Table 1. Average cell growth and growth rates of *B. plicatilis* at 30‰ salinity concentration

Days	The number of rotifer cells ($10^5 \times \text{rot/mL}$)	$K = \mu$ (specific growth rate)
	$\bar{X} \pm S_{\bar{X}}$ (N=3)	$\bar{X} \pm S_{\bar{X}}$ (N=3)
1	$0,170 \times 10^5 \pm 0,0000$	$0,000 \pm 0,000$
2	$0,247 \times 10^5 \pm 0,0136^*$	$-1,396 \pm 0,0557^*$
3	$0,413 \times 10^5 \pm 0,0404^*$	$-0,503 \pm 0,1737^*$
4	$0,716 \times 10^5 \pm 0,0557^*$	$-0,558 \pm 0,769^*$
5	$1,600 \times 10^5 \pm 0,1552^*$	$0,149 \pm 0,0978^*$
6	$1,286 \times 10^5 \pm 0,5217^*$	$0,230 \pm 0,2480^*$
7	$0,966 \times 10^5 \pm 0,0351^*$	$0,229 \pm 0,0351^*$
8	$1,000 \times 10^5 \pm 0,0700^*$	$0,032 \pm 0,0235^*$
9	$1,328 \times 10^5 \pm 0,2054$	$0,556 \pm 0,4383^*$
10	$1,347 \times 10^5 \pm 0,1151^*$	$0,507 \pm 0,3175^*$
11	$1,388 \times 10^5 \pm 0,3067^*$	$0,152 \pm 0,2127^*$
12	$1,463 \times 10^5 \pm 0,3821^*$	$0,111 \pm 0,2802^*$
13	$1,997 \times 10^5 \pm 0,1753^*$	$0,256 \pm 0,0976^*$
14	$2,039 \times 10^5 \pm 0,0686^*$	$0,136 \pm 0,0350^*$
15	$2,04 \times 10^5 \pm 0,0321^*$	$0,036 \pm 0,0155^*$

*The difference between the previous day and the next day is important in terms of cell increase on the basis of the colon ($p < 0,05$).



Graphic 1. Average cell growth and growth rates of *B. plicatilis* at 30‰ salinity concentration

4. Discussion and Conclusion

The experiment was carried out in a helical photobioreactor in a continuous system. In the continuous system, the inflow of algae into the rotifer tank was adjusted by diluting according to the specific growth rate of the algae, and the rotifer was harvested daily from the culture dish for 24 hours. In this way, it was possible to obtain the rotifer at maximum growth performance by providing continuous fresh algae inflow and continuous rotifer exit from the environment. In addition, since there is no pollution in the rotifer tank, it was possible to harvest high quality rotifers in a long period (15-90 days). During the experiment, the initial density in the continuous system production of rotifer was 0.170×10^5 rotifer/L, the first five days were with batch production, and the production

continued with the continuous system from the 5th day. The increase in rotifers, respectively, was in the range of $1,750 \times 10^5$ - 2.053×10^5 rotifer/L between the 5th day and the 15th day, and the growth performance increased 10 times on average.

The fact that the highest individual increase for rotifers was obtained at 30‰ salinity was consistent with the information that the optimum salinity for rotifers was 30‰ (Pechmanee 1988; Hagiwara & Hino 1990; Chen 1991). Optimum salinity concentrations for *Isochrysis galbana* and *Nannochloropsis oculata* species in China were determined as 28‰ and 4-36‰, respectively (Chen & Long 1991). The salinity concentrations at which the highest cell numbers were detected are close to the optimum values specified by various researchers. En yüksek hücre sayılarının saptandığı tuzluluk derişimleri çeşitli araştırmacılar tarafından belirtilen optimum değerlere yakındır. As a result, it can be recommended to adjust the salinity concentration as 30‰ in *Nannochloropsis oculata* cultures and 20‰ in *Isochrysis galbana* cultures in order to increase the number of algal cells and achieve a higher growth rate. In our study, it is confirmed by the continuous increase of *B. plicatilis* from the beginning at 30‰ salinity concentration and the increase in cells between the 11th day and the 15th day as 1.736×10^5 - 2.053×10^5 rot/L. According to Chen & Long (1991); In order to increase the number of algal cells and achieve a high growth rate, it is recommended to determine the salinity concentration as 30‰ in *N. oculata* cultures and 20‰ in *I. galbana* cultures. Average number of rotifers and growth rates of *B. plicatilis*, 9.-10. days $1,328 \times 10^5$ - $1,347 \times 10^5$ rotifer/L; average growth rates of 0.556-0.507 divisions/day were determined at high values. The highest mean number of rotifers on the 14th day was 2.039×10^5 rotifer/L; growth rates were determined as 0.136 divisions/day. The highest mean number of rotifers on day 15 was 2.040×10^5 rotifer/L; growth rates are 0.036 divisions/day, respectively. Pozuela & Lubian (1993) stated that the growth rate in rotifers was 0.456 /day at 25‰ salinity, 0.294 /day at 40‰ salinity, and the growth rate for *B. plicatilis* at low and medium salinities gave better results. According to the results obtained, the growth rate of rotifers fed with *Nannochloropsis oculata* on the 9th day at 30‰ salinity is 0.244-0.346 divisions/day. It was determined as 0.156- 0.203 divisions/day on the 14th day and 0.020-0.067 divisions/day on the 15th day. These data coincide with the study of Pozuela & Lubian (1993). Hindioğlu (1995) examined the increase of rotifers in *B. plicatilis* (20‰-35‰) and stated that low salinities gave the best results and that there were statistically significant differences between salinities in growth rate.

James and Rezeq (1988) 0.16±0.01 /day at 30‰ salinity, 59.33±8.54 rot./ml/day, Rezeq & James (1987) 0.193 at 30‰ salinity /day, 36.33±1.53 rot./ml/day. According to the data obtained in our study, the highest growth rate on the 14th day was determined as 0.156- 0.203 division/day. James & Rezeq (1988); Rezeq & James (1987) agree with the work of the researchers. The highest rotifer number was obtained at 30‰ salinity, which is similar to the information that the optimum salinity for rotifers is 30‰ (Pechmanee 1988; Hagiwara & Hino 1990; Chen 1991). It has been determined that it is the best food that provides population growth in rotifer culture in *N. oculata* algae species at 25‰ salinity (Savaş & Güçlü 2004).

5. Conclusion

N. oculata algae is one of the most important nutrients used in the production of rotifer *B. plicatilis* (Spolaore *et al.* 2006). The use of the small, live bait *B. plicatilis* is still a prerequisite for success in hatcheries of marine fish larvae such as sea bream and flatfish (Spolaore *et al.* 2006). Still, more research is needed on the effect of such rotifers on the production of fish and/or crustaceans. However, little is known about the effect of the nutrient environment on microalgae, as well as the effect of such microalgae on rotifer production performance and nutritional content (Campaña-Torres *et al.* 2012). Moreover, the production quality of rotifers as a food source depends not only on the microalgae species they consume, but also on the method of feeding. Considering all these issues, as a continuation of this study, it is necessary to look at the nutritional content and egg productivity of rotifer *B. plicatilis* produced in a helical photobioreactor by feeding with different algae species

As reported by the researchers, it is recommended to conduct more extensive studies on the effects of different salinity concentrations on the population growth and growth rates of rotifers.

As a result, in the study; the algae produced at maximum density in the continuous system in the helical photobioreactor were given to the *B. plicatilis* tank and harvested daily from the rotifer culture tank for 24 hours. During the experiment, the growth performance of *B. plicatilis* increased by an average of 10 times. It is thought that the results of this study can be evaluated in order to increase the production of rotifers in marine fish farms where larvae are produced, and while ensuring continuity in rotifer production, an increase in product productivity will be achieved.

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