

Original Article

**Effect of Un-live Microalgal diet, *Nannochloropsis oculata* and *Arthrospira (Spirulina) platensis*, Comparing to Yeast on Population of Rotifer, *Brachionus plicatilis***

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**Abstract**

Rotifers *Brachionus plicatilis* are the most common zooplankton used to rear early larvae of marine shrimp and finfish. Reduce production cost of live food in marine hatcheries is considered one of the main targets for developing a marine aquaculture industry. The present study is conducted to investigate the population (as increase in number) and population growth rate of rotifer *Brachionus plicatilis* fed on un-live microalgal diet, frozen *Nannochloropsis oculata* (FN) and dried *Spirulina platensis* (DS), comparing to dried baker's yeast *Saccharomyces cerevisiae* (Y) in four treatment; alone for each diet and mixing between *S. platensis* + yeast and yeast: (FN, DS, DS+Y, and Y). The experiment was continued for 12 day in batch culture system with initial rotifer population 100/ind./ml. The lowest average in population and population growth rate were observed in rotifer fed on SD (122.90±43.38 Ind./ml and 0.137 ind./day, respectively), while the highest  $R^{FN}$  and  $R^Y$  were observed in Y (182.24±43.89 ind./ml and 0.569 ind./day), followed by DS + Y (166.00 ± 32.58 Ind./ml and 0.486 ind./day) and FN (130.09 ± 21.51 ind./ml and 0.251 ind./day, respectively). In general, Baker's yeast is cheap and readily available; in contrast, microalgae (fresh, freeze-dried) are laborious, time consuming and expensive. Baker's yeast *S. cerevisiae* has also been successfully used for rotifers with high population and population growth rate.

**Key words:** Rotifer, *Brachionus plicatilis*, *N. oculata*, *S. platensis*, yeast.

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# Effect of Un-live Microalgal diet, *Nannochloropsis oculata* and *Arthrospira (Spirulina) platensis*, Comparing to Yeast on Population of Rotifer

## Introduction

Production of live food is considered a key component of the productive process in marine hatchery, remains one of the most important obstacles for the development of marine aquaculture (Jabeur et al., 2013). *Brachionus* is a complex zooplankton species and at least three sympatric species (*Brachionus plicatilis*, *Brachionus rotundiformis* and *Brachionus ibericus*) have been observed. The two species *B. plicatilis* and *B. rotundiformis* are used worldwide to rear early developmental stages of marine shrimp and finfish. It is easily digestible, has appropriate size, can survive in high stocking densities and swims slowly which giving an ample opportunity to its predator for prey (Lubzens et al., 2001). However, among them *B. plicatilis* was widely used as live feed in marine aquaculture (Qie et al., 2011). Despite attempts to develop artificial micro-diets, live feed organisms are still essential for culturing marine larval fish. Rotifers are selective feeders and may be cultured and grown on a number of diets. Rotifers have little nutritional value, called as a nutrient container, and get the essential nutrient from his diets. Lubzens et al. (2001) stated that, to the grower of marine shrimp and fish larvae, rotifers are live food capsules that deliver essential nutrients for survival and growth of marine larvae. This means that the nutritional value of rotifer for marine larvae depends on the rotifers food source. Rotifers actively graze the water column feeding on particles approximately 1 to 30 microns in sizes. Apart from pure algae, there is a number of yeast or algae-based rations suitable for culturing rotifers that are commercially available (Chew and Lim, 2006). Rotifers are fed on different microalgal species, such as *Nannochloropsis*, *Chaetoceros*, *Tetraselmis*, *Dunaliella* (Abdel Rahman et al., 2008), *Spirulina*, *Chlorella* (Jabeur et al., 2013 ) and *Pavlova* (Hemaiswarya et al., 2011), as live, frozen, or dried microalgae. The high cost of microalgae may limit rotifer production and success in marine hatcheries. A single fish larva may require more than 40,000 – 100,000 rotifers before it is weaned to another diet. Therefore, the production demand of rotifers is extremely high in order to support marine fish culture

(Lind., 2014) Since Hirata and Mori (1967) introduced the use of bakers' yeast *Saccharomyces cerevisiae* as food for marine rotifer *B. plicatilis*, number of investigators, researchers and aquaculture have been used bakers' yeast as food for this species; rotifers grown in this way have also been nutritionally enriched. Moreover, oil enrichment, artificial diets, baker yeast and baker's yeast with added marine oils may be used as rotifer diets as worldwide cheap food for the production of *B. plicatilis* (Ferreira, et al., 2009). There are advantages and disadvantages in using each of algae and yeast in culturing rotifer (Lind., 2014). However, baker's yeast is a cost effective substitute for algal-based diets for rotifers. Yeast is ten times less expensive than microalgae diets, and rotifers can be successfully grown on yeast alone (Nagata 1989). Despite rotifer feed on baker's yeast only may consider poor in their nutritional value, especially DHA and EPA, rotifer may be enriched with nutrient elements to enhance their nutritional value, regarding to increase the survival and growth of marine larvae. Microalgae diets helps preserve good water quality in the rotifer culture system and enhance rotifer biochemical composition, but microalgae is laborious, time consuming and expensive and has a shorter shelf life (Arnold and Holt, 1991; Lind., 2014). Still, a combination of yeast and algae is preferred over yeast alone for mass rotifer production as mentioned above (Hache and Plante, 2011). The aim of the present study is comparing the effect of baker's yeast (dry form) and two un-live microalgae, common used as rotifer diet, in frozen (*N. oculata*) and dried form (*S. platensis*) and mixing of them, on population and population growth rate of rotifer *B. plicatilis*.

## MATERIALS AND METHODS

The present study was conducted in El-Max Research Station, Alexandria Branch, Aquaculture Division, National Institute of oceanography and Fisheries (NIOF). *N. oculata* was obtained, as freeze-dried cells, from Microalgae Culture Room, Invertebrate Aquaculture Lab., Alexandria Branch, Aquaculture division, (NIOF).

### Microalgae and Yeast

Two microalgal species were used in this experiment, freeze-dried *Nannochloropsis oculata* and dried *Spirulina platensis* (*Arthrospiraplantensis*), and yeast *Saccharomyces cerevisiae*. *Spirulina platensis* was cultured as batch culture in plastic jars (16 L) and harvested (at density  $14.5\text{-}19.8 \times 10^6$  cell/ml) by centrifugations (300 rpm/5 min.), and finally saved as freeze-dried cells ( $-20^\circ\text{C}$ ) until used for rotifer feed experiment. *S. platensis* were obtained as dry powder from (Argent Company, USA). The biochemical composition of dry powder of *S. platensis* were protein (55%), lipid (4%), fiber (7%), ash (7%), moisture (7%) and the total carotenoids was 300mg/100g. Beside microalgae, the dry yeast *S. cerevisiae* (Starch and Yeast Co., Egypt) was used as feed for rotifer *B. plicatilis*.

### Rotifer Experimental Design

*Brachionus plicatilis* (L-type with average length about 180  $\mu\text{m}$ ) were used at density 100 ind./ml (Yamasaki, et al., 1989) and kept during the experiment under controlled culture conditions of temperature  $26.5 \pm 1^\circ\text{C}$ , salinity  $25 \pm 1$  ppt, and pH  $7.8 \pm 0.2$ . The rotifer *B. plicatilis* feeding experiment was continued for 12 day in batch culture system. In this experiment, four treatments were conducted in jars 16 liters, three replicates for each treatment. The rotifer

diet regimes were added to each jar as the following; (1) FN: Freeze-dried *N. oculata* ( $1.5 \text{ g}/10^6$  ind./day), (2) DS: Dried *S. platensis* ( $1 \text{ g}/10^6$  ind./day), (3) DS+Y: dried *S. platensis* ( $0.5 \text{ g}/10^6$  ind./day) + yeast *S. cerevisiae* ( $0.5 \text{ g}/10^6$  ind./day), and (4) Y: baker's yeast *S. cerevisiae* ( $1 \text{ g}/10^6$  ind./day). The rotifer diets (microalgae and yeast) were added in rotifer jars every day in two times; in the morning and in the afternoon. Each time of add was containing half of previously quantities.

### Tested Parameters

#### Population ( $R^{FN}$ )

During the experiment (12 day), the populations of rotifer ( $R^{FN}$ , calculated as increase in number) were determined each 48 hours by counting the individuals of rotifer (five times) in a 1-ml glass pipette using Sedgwick-Rafter cell binocular microscope.

#### Population Growth Rate ( $R^f$ )

The population growth rate of rotifers ( $R^f$ ) was calculated according to Yin et al., (2013) using the following equation:

$$r = (\ln N_t - \ln N_0)/t$$

Where;  $N_0$  and  $N_t$  are the initial and final population densities, and the incubation time in days ( $t$ ).

### Statistical analysis

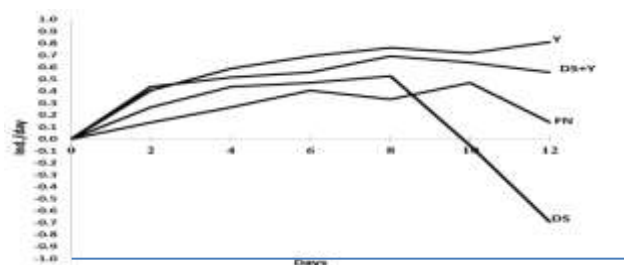
Statistical analyses were performed using analysis of variance (One-way ANOVA) to determine significant differences exist in rotifer population between treatments. Differences among means were considered significant at  $p < 0.05$  multiple range of post hoc, and comparisons were performed using the least significant difference (LSD) to resolve the differences among the means of replication according to Duncan, (1955) using SPSS (1997), version 17.0.

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### RESULTS

Table (1) showed the population ( $R^{FN}$ ) and population growth rate ( $R^f$ ) of *B. plicatilis* fed on freezed *N. oculata* (FN), dried *S. platensis* (DS), mixing between dried *S. platensis* and dried yeast (DS+Y), and dried yeast (Y). Fig. 1 showed the population growth rate ( $R^f$ ) of rotifer fed on different microalgal diets. There are significant differences were observed between all treatment used in the experiment. The lowest average in  $R^{FN}$  and  $R^f$  were observed in SD ( $122.90 \pm 43.38$  ind./ml and 0.137 ind./day, respectively), while the highest  $R^{FN}$  and  $R^f$  were observed in Y ( $182.24 \pm 43.89$  ind./ml and 0.569 Ind./day), followed by DS+Y ( $166.00 \pm 32.58$  ind./ml and 0.486 ind./day) and FN ( $130.09 \pm 21.51$  ind./ml and 0.251 ind./day, respectively), as shown in Fig. 2.

After eight days left from beginning of the experiment, the highest significant  $R^{FN}$  and  $R^f$  were achieved in Y ( $214.67 \pm 14.64$



**Figure 1.** Effect of different diets on rotifer population and population growth rate.

FN: freezed *N. oculata*, DS: dried *S. platensis*, FN+DS mixing between freezed *N. oculata*, and dried *S. platensis*, and Y: dried yeast.

### DISCUSSION

The present study concerning on the population (as increase in number) and the population growth rate of rotifer *B. plicatilis* fed on dried baker's yeast *S. cerevisiae*, comparing with the two un-live microalgal species, *N. oculata*, in the freezed form, and *S. platensis*, in the dried form, as a single form, and mixture diet, in equal proportion,

ind./ml and 0.764 ind./day), DS+Y ( $200.33 \pm 18.18$  ind./ml and 0.695 ind./day), and DS ( $169.67 \pm 14.57$  ind./ml and 0.529 ind./day), while FN achieved the highest significant  $R^{FN}$  and  $R^f$  ( $130.09 \pm 21.51$  ind./ml and 0.251 Ind./day) after ten day left from beginning of the experiment. As well as, after 12 day of the experiment, the highest significant  $R^{FN}$  and  $R^f$  was observed in Y ( $225.33 \pm 11.01$  ind./ml and 0.812 Ind./day). In Y treatment, the peak of  $R^{FN}$  and  $R^f$  still raise during the end of experiment, while the peak was breakdown in FN after ten days, and after ten days in treatments DS + Y and DS. The breakdown of  $R^{FN}$  and  $R^f$  still higher than the initial of the experiment in FN and DS + Y, while the breakdown exceeded initial of  $R^{FN}$  and  $R^f$  in the beginning of the experiment, either in after ten days ( $95.00 \pm 7.00$  ind./ml and - 0.051 ind./day) or twelve days ( $50.00 \pm 11.00$  ind./ml and - 0.693 ind./day).

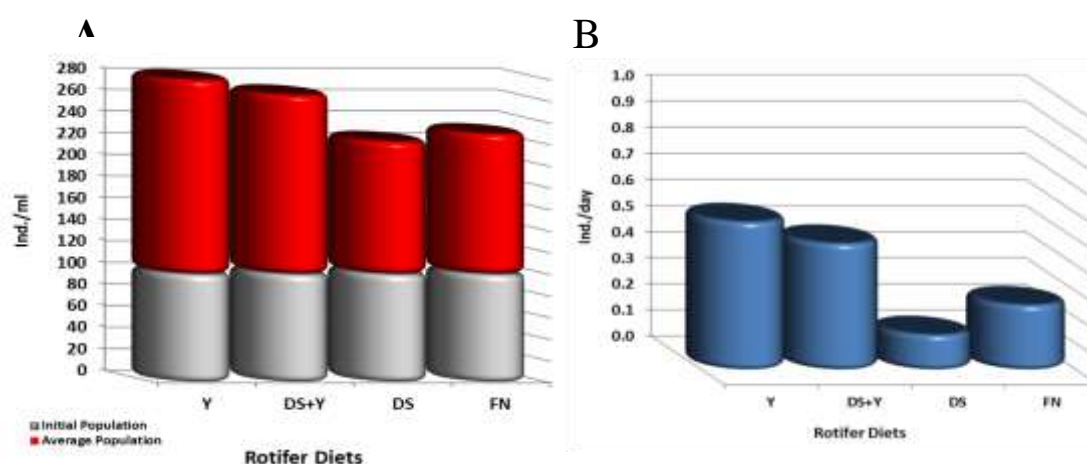
between *S. platensis* and yeast. However, during all days of the experiment, the population and population growth rate of rotifer fed on baker yeast *S. cerevisiae* were higher than those fed on un-live microalgal species (freezed *N. oculata* and dried *S. platensis*). However, Tamaru (1993) cited that rotifers fed only yeast are subject to potential stagnation in rotifer growth; however, high growth can be achieved by feeding rotifers a combination of yeast and algae.

Our results in the same line with (Nhu 2004), who studied the effects of different four different diets: (1) bakers' yeast (*Saccharomyces cerevisiae*) in wet form plus 10% squid liver oil (by dry weight), (2) *S. cerevisiae* in dry form plus 10% squid liver oil, (3) live microalgae *Nannochloropsis oculata* and (4) live microalgae *Chaetoceros muelleri*, on growth and quality of rotifer of *B. plicatilis*. The results showed that there were significant differences in rotifer growth rate between the four dietary treatments.

**Table 1.** Effect of different diets on population and population growth rate of rotifer

Days	FN		DS		DS+Y		Y	
	$R^{FN}$	$R^r$	$R^{FN}$	$R^r$	$R^{FN}$	$R^r$	$R^{FN}$	$R^r$
0	100.00±0.00 <sup>d</sup>	0.000	100.00±0.00 <sup>c</sup>	0.000	100.00±0.00 <sup>d</sup>	0.000	100.00±0.00 <sup>d</sup>	0.000
2	115.00±5.00 <sup>cd</sup>	0.140	130.33±16.25 <sup>b</sup>	0.265	155.00±26.63 <sup>c</sup>	0.438	150.00±25.00 <sup>c</sup>	0.405
4	130.33±16.62 <sup>bc</sup>	0.265	155.00±26.63 <sup>ab</sup>	0.438	167.67±14.19 <sup>bc</sup>	0.517	180.33±9.5 <sup>bc</sup>	0.590
6	150.33±27.93 <sup>ab</sup>	0.408	160.33±18.01 <sup>a</sup>	0.472	174.67±14.05 <sup>abc</sup>	0.558	200.33±13.43 <sup>ab</sup>	0.695
8	139.67±14.84 <sup>abc</sup>	0.334	169.67±14.57 <sup>a</sup>	0.529	200.33±18.18 <sup>a</sup>	0.695	214.67±14.64 <sup>a</sup>	0.764
10	160.33±18.01 <sup>a</sup>	0.472	95.00±7.00 <sup>c</sup>	-0.051	189.67±7.64 <sup>ab</sup>	0.640	205.00±13.23 <sup>ab</sup>	0.718
12	115.00±3.00 <sup>cd</sup>	0.140	50.00±11.00 <sup>d</sup>	-0.693	174.67±14.01 <sup>abc</sup>	0.558	225.33±11.01 <sup>a</sup>	0.812
Aver.	130.09±21.51	0.251	122.90±43.38	0.137	166.00±32.58	0.486	182.24±43.89	0.569

The data were means±SD ( $P < 0.05$ ) of population ( $R^{FN}$ ) and population growth rate ( $R^r$ ) of *B. plicatilis*. **FN**: freeze-dried *N. oculata*, **DS**: dried *S. platensis*, **DS+Y** mixing and dried *S. platensis* and yeast, and **Y**: dried yeast.



**Figure 2.** Effect of different diets on the average of rotifer population (A) and population growth rate (B). **FN**: freeze-dried *N. oculata*, **DS**: dried *S. platensis*, **FN+DS** mixing between freeze-dried *N. oculata*, and dried *S. platensis*, and **Y**: dried yeast.

Moreover, rotifers fed on microalgae showed better viability, larger size and low ciliate contamination compared to those fed on yeast. However, higher density and production of rotifers were found with the use of yeast, but there was a problem in controlling the ciliate contamination and maintaining the cultures.

Pena-Aguado et al., (2005) studied the population growth rate of two rotifer *B. calyciflorus* and *B. rubens* fed on three diets (*Chlorella vulgaris* (Ch), *Scenedesmus acutus* (Sc) and yeast (Y) in seven combinations (alone or mixed in equal proportions, on dry weight basis): Ch+Sc,

Ch+Y, Sc+Y and Ch+Sc+Y). The population growth curves of *B. calyciflorus* and *B. rubens* revealed that algal diets were superior to yeast. Regardless of diet, *B. rubens* had a longer lag phase and delayed peak density compared to *B. calyciflorus*. In general, he concluded that yeast could effectively supplement algal diets in all the test species, thereby reducing costs in large scale production of rotifer. On the other hand, it is also not known whether different *Brachionus* species show different population growth rates when grown on yeast (Sarma et al., 2001). While it is important to consider rotifer growth and

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cost of the diet, it is equally important to understand the diet's effect on the culture's microbial community (Lind., 2014). Unfortunately, using of yeast in rotifer culture may cause contaminations by ciliated protozoa and bacteria. On the other hand, various commercial microalgae diets are available on the market but differ in rotifer growth and associated microbial communities (Qi et al., 2009).

Biochemical composition of rotifer fed on yeast and microalgae is important point to discuss. Compared to microalgae, yeast is deficient in omega-3 amino acids and other essential nutrients. Striped mullet and milkfish fed rotifers grown on yeast have lower survival than fish fed rotifers that are grown on microalgae or a combination of microalgae and yeast. Ferreira et al., (2009) reported that *Nannochloropsis sp.* is widely used for rotifer production since it supports higher growth rates than baker's yeast and provides the rotifers with high amounts of EPA. Moreover, microalgae like *Nannochloropsis*, *Tetraselmis*, *Dunaliella*, and *Chlorella*, are deficient in DHA, and feeding them to *B. plicatilis* results in DHA/EPA ratios lower than 0.5. HUPA-rich microalgae such as *Isochrysis* and *Pavlova* contain high DHA levels and can be fed to *B. plicatilis* for DHA enrichment, which results in DHA/EPA ratios above 2.0 (Hemaiswarya et al., 2011). However, the nutritional value of baker's yeast (as not laborious, not time consuming and not expensive diet for rotifer) may be enhance easily by adding of marine oil enrichment, especially EPA and DHA that are needed for all marine larvae (Ferreira, et al., 2009).

The microalgae form (live, frozen and dried) as rotifer diets is another interesting point to discuss. Using of live form of marine microalgae, especially *N. oculata*, as fresh rotifer diet is well known in giving high population and population growth rate of rotifers, when comparing with dry baker's yeast. Convert live microalgae to freeze or dry form is important in marine hatcheries which may resolve the culture problems

during the hatching season, like the decrease of culture and contamination by ciliated protozoa. Correct treatments in freezing or drying of microalgae do not make microalgal cells lose most of their nutritional value. On the other hand, convert the fresh microalgal diet form may solve the problem of contaminations by ciliated protozoa and make it as freed or dried organic matter diet for rotifer or marine larvae.

In the present work, we used the baker's yeast, without enrichment with any marine oils, as rotifer diet in comparing to converted microalgal cells (frozen *N. oculata* and dried *S. platensis*) to investigate the effect of microalgal form on rotifer population. Dried baker's yeast achieved the highest rotifer population during all experiment days which make baker yeast an optimal rotifer diet comparing to frozen and dried microalgae.

### Conclusion

Reduce production cost of rotifer *B. plicatilis* in marine hatcheries is considered one of the main targets for developing a marine aquaculture industry. Baker's yeast is cheap and readily available; in contrast, microalgae (fresh, frozen and dried) are laborious, time consuming and expensive. Baker's yeast *S. cerevisiae* has also been successfully used for rotifers with high population and population growth rate.

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