

Original Research Article

Effect of different diets on the growth rate of the rotifer, *Brachionus plicatilis* under 40 ppt salinity stress.

Comment [D1]: You said that "Rotifers are cosmopolitan organisms as they are osmoconformers and can be adapted to salinities ranging from 1‰ to 97‰". Thus, how do you know rotifer to be stressed as cultured under 40ppt? Not found any stress conditions or challenging test carried out in this manuscript?

ABSTRACT

Aquaculture is important business some fish farms use underground water that may has high salinity. The aim of this study is to evaluate the effect of eight different types of diets on enhancing the growth rate of the rotifer, *Brachionus plicatilis* lives in 40 ppt saline water. The diets used in the study are T1 (*Nannochloropsis oculata*), T2 (*Tetraselmis suecica*), T3 (*N. oculata* + *T. suecica*), T4 (*N. oculata* + yeast; *Saccharomyces cerevisiae*), T5 (*T. suecica* + yeast), T6 (*S. parkle*), T7 (*S. Parkle* + yeast) and T8 (yeast). The results revealed that the rotifer fed for *S. parkle* showed the highest growth population (818 Indv. /mL) with a highly significant difference ($p < 0.05$). On the other hand, rotifers fed the yeast (*S. cerevisiae*) showed the lowest population growth (355 Indv. /mL). The outcome of the current study indicates that *S. parkle* commercial diet is very appropriate for the stock culture of *B. plicatilis* due to its ability to increase the growth rate of the rotifer in a short time under the current conditions.

Keywords: *S.parkle*, *Yeast*, *Tetraselmis suecica*, *Nannochloropsis oculata*, *Zooplankton*, *Fish food*

1. INTRODUCTION

Zooplankton is the first food for many fish crops due to its effectiveness in increasing the growth rate of fish larvae. It is also considered as an easily digestible protein for fish and shellfish (EL-Kassas *et al.*, 2015). Rotifers as a zooplankter is used as natural food for larvae (Ichthyoplankton) in hatcheries as they are the favorite prey of most fish larvae (Pfejffer and Ludwig, 2007).

One of the vital difficulties faced production of fish fry in hatcheries is the lack of live food for the nutrition of early stages of fish larvae. Therefore, there is no alternative food that could replace the using of rotifers as preliminary food for larvae until now (Hagiwara *et al.*, 2001; Yoshimatsu and Hossain 2014). Moreover, the rotifer, *Brachionus plicatilis* is an essential

food type especially in the early larval stages of many fish species due to its small size and its ability to propagate in captivity at high density and reproductive rate (Alam and Shah, 2004). Additionally, it has the ability to stand wide ranges of salinity (Abd Rahman *et al*, 2018).

Furthermore, rotifers need different types of food to survive and be active in the hatcheries. Various species of microalgae have been used as food for rotifers including; *Tetraselmis* spp., *Nannochloropsis* spp., *Chaetoceros* spp., *Rhodomonas* spp. and *Isochrysis* spp. (Dhert *et al*. 2001; Hoff and Snell 2001; and Wikfors and Ohno, 2001). In contrast, baker's yeast, *Saccharomyces cerevisiae* is immediately available compared to the microalgae (fresh, frozen or dried) which are laborious, time-consuming and expensive. Additionally, the yeast, *S. cerevisiae* has an important role to give a high population growth rate for rotifers (Heneash *et al*, 2015). However, *Nannochloropsis* sp. is widely used as live food for rotifers as it provides the predicted yield on time with high density because of its ease of cultivation (Abd Rahman *et al*, 2018).

In Egypt, El-Wafa fish farm is located in the Ismailia governorate next to the Suez Canal and the groundwater well is the source of aquaculture water, the well's salinity is 40 ppt. The farm produces *Dicentrarchus labrax* and *Sparus aurata*. Consequently, during the feeding of the grown fish with natural food (zooplankton), species of zooplankton that tolerate salinities above normal sea water (35-37 ppt). Hence, the present work aims to study the effects of 40 ppt salinity on the rotifer (*B. plicatilis*) growth on different feeding diets and the effect of an artificial food type and initial stocking density on the population growth of rotifers to induce high productivity of *B. plicatilis* to be used as fish food.

Comment [D2]: Why the aim is different from the title. Do you want to study on the effect of salinity or the diets?

2. MATERIAL AND METHODS

The experiment was conducted in El-Wafa fish farm, Ismailia, Egypt. *Brachionus plicatilis* specimens were obtained from the marine hatchery of the National Institute of Oceanography and Fisheries (NIOF), Alexandria, Egypt. The rotifers were divided into eight groups each one has three replicates. Then, they were stocked in twenty-four 9- liters plastic jars supplied with continuous aeration. Water parameters were measured daily including water temperature (digital thermometer), water acidity using Jenway pH meter, (Model 350) and salinity using digital salinometer. Two marine green microalgae were tested; *Nannochloropsis oculata* and *Tetraselmis suecica*. Each algal species were then combined with the baker's yeast (*Saccharomyces cerevisiae*) and Selco S. parkle; which is yeast-based ready commercial diet for rotifers manufactured by INVE.

Dietary treatments:

Eight diets are (T1-T8), where T1 (*N. oculata*) was considered as a control, T2 (*T. suecica*), T3 (*N. oculata* + *T. suecica*), T4 (*N. oculata* + yeast; *S. cerevisiae*), T5 (*T. suecica* + yeast), T6 (S.parkle), T7 (S.parkle + yeast) and T8 (yeast).

Experimental procedure

The experiment started with two liters initial volume containing 150 indiv./L initial rotifer density for each replicate in each treatment. They were supplied with the indicated diet (Table 1) twice daily at 09.00 AM and 14.00 PM in 1000 mL saline water (underground water) for 8 days.

Comment [D3]: 150 indiv/L or 150 indiv/mL

Comment [D4]: Not clear. Why do you set up twenty four 9-liters plastic jar, but using only 2-liter initial volume and in the Table 1 you showed only 1-liter initial volume?

Table 1: The eight diets of feeding diets and their feeding rates/jar/day.

Treatment	Feeding diets	Initial volume of water/Jar 1 st day	Feeding rate/Jar/day
T1	<i>Nannochloropsis oculata</i>		1000 mL
T2	<i>Tetraselmis suecica</i>		1000 mL

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Comment [D5]: If you applied feeding regimes following Table 1, density of rotifer in T1, T2...T5 were diluted that affecting your result.

T3	<i>N. oculata</i> + <i>T. suecica</i>	1000 mL*	1000 mL
T4	<i>N. oculata</i> + Yeast (<i>Saccharomyces cerevisiae</i>)		1000 mL+ 0.1 gm/l
T5	<i>T. suecica</i> + Yeast (<i>S. cerevisiae</i>)		1000 mL+ 0.1 gm/l
T6	S.parkle		0.1 gm/l * 50 mL
T7	S.parkle + Yeast (<i>S. cerevisiae</i>)		0.1 gm/l * 50 mL
T8	Yeast (<i>S. cerevisiae</i>)		0.1 gm/l * 50 mL

* Each one mL contains 150 indiv. /l

Sampling and counting of *B. plicatilis*

Each zooplankton density in each treatment zooplankter was determined using a binocular microscope (40X). The density of rotifer organisms was calculated as their total number per cubic meter. The concentrated rotifers were subsampled using a Stempel pipette (Heneash, 2015; El-Damhogy *et al.*, 2016; Aboul Ezz *et al.*, 2014; El-Kassas *et al.*, 2015).

Population (R)

Along the eight experimental days, the population of rotifer was calculated as the increase in number every 24 hours by counting the individuals (three times) in a 1-mL glass pipette. These concentrated rotifers were sampled with a Pasteur pipette and were counted under the dissecting microscope (Braley, 1994).

Population Growth Rate (R^r)

The population growth rate of rotifers (R^r) was calculated according to Okauchi and Fukusho (1984) using the following equation:

$$R^r = \frac{(N_t - N_0)}{t}$$

where R^r = the instantaneous growth rate; N_0 = the initial number of rotifers; N_t = the final number of rotifers after t days; t = culture days.

Statistical analysis

Data were analyzed using analysis of variance test (One-way ANOVA) to determine the significant differences among rotifer populations between treatments. Also, Duncan's multiple range test was used to provide more information of ranges of all the pairs of treatments using IBM SPSS Statistics (v22).

3. RESULTS

Water quality

During the experiment period, the pH value was 8.0 ± 0.2 . Water temperature and salinity were $28.0 \pm 2.0^\circ\text{C}$, 40 ± 1.0 ppt, respectively.

Comment [D6]: Why do you use cubic meter unit?, while whole your manuscript had used indiv./ml

Comment [D7]: Not uniform font

Comment [D8]: The counting method in the Sampling and Population section is totally different, why?

Effect of different diets on the growth rates of *B. plicatilis* during the experiment period.

During the eight experiment days, T6 (S. parkle) was the most effective diet which gave the maximum average density of *B. plicatilis*, (818 Indv. /mL). On the other hand, T8 was the least effective diet which contributed the minimum average density represented only by (355 Indv. /mL). Figure (1) shows the impact of the eight experimental diets studied during the experiment period, indicating that the highest average value listed in the ninth day was (935 Indv./mL), while the lowest average value recorded in the first day was (265 Indv./mL). Besides, the aforementioned figure revealed that T6 was the most effective diet which gave a distinct increase in the growth rate of *B. plicatilis*.

Comment [D9]: Where is the data on the ninth day?

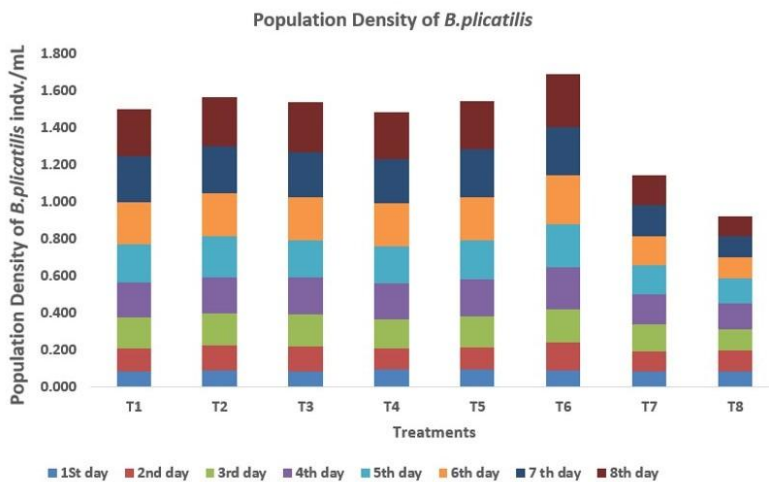


Figure1: Effect of the eight experimental diets during the experiment period.

Furthermore, Table (2) revealed that there were highly significant differences among the eight experimental diets where the significance level of ($F = 2.665, p = 0.019$) is less than the threshold value of ($p < 0.05$). In conclusion, not all the group means were equal which revealed that the effects of treatments were not similar in the present experiment.

Comment [D10]: What is your mean here? What is "not all the group mean were equal"??

This leads to the fact that at least one of the groups tested differs from the other. In addition, Duncan's multiple range test was used to determine the critical values for comparisons among means. The test revealed that the treatment pairs T1 and T3, T1 and T4, T1 and T5, T1 and T6, T1 and T7, T1 and T8, T2 and T5, T2 and T6, T2 and T7, T2 and T8, T3 and T6, T3 and T7, T3 and T8, T4 and T7, T4 and T8, T5 and T7, T5 and T8 had mean differences with significant value ($p < 0.05$). The rest pairs of treatments showed a significance level greater than (0.05) which means there were no statistically significant differences between them.

Comment [D11]: You should be made a Table that is easy to compare all your treatments. This Table can replace Table 2, which is not showing differences among the treatments.

Table 2: Results of the one-way ANOVA test for the population density of rotifer for the eight experimental diets.

Sources of Variation	Sum of Squares	df	Mean Square	F	p-value
Between Groups	0.057	7	0.008	2.665	0.019
Within Groups	0.170	56	0.003		
Total	0.227	63			

The pairwise comparison among treatments showed a highly significant difference in T6 (*S.parkle*) and T8 (*S. cerevisiae*) where p -value = 0.0015 (Figure. 2).

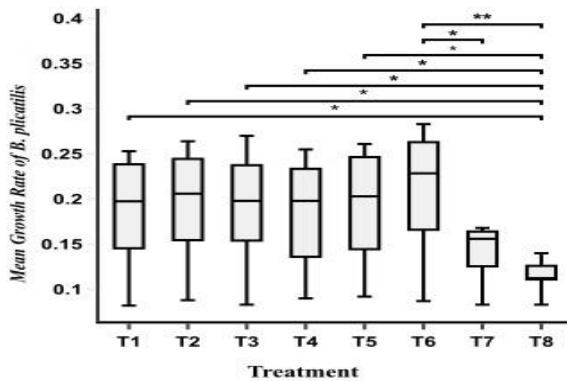


Figure 2: Boxplot provides a pairwise comparison between the experimental treatments (Duncan's multiple range test), the number of asterisks depends

upon the p -value of particular pair of samples; * for $p < 0.05$ and ** for $p < 0.01$.

Gravid females of *B. plicatilis*

The gravid females of *B. plicatilis* (Indv. /mL) during the Experiment period was shown in Figure (3). Treatment 4 displayed the greatest average number of gravid females, (144 Indv. /mL) which began with (96 Indv. /mL) on the first day of the experiment. In contrast, the lowest average number of the gravid females of the studied rotifers was for T7 (47 Indv. /mL) which started by (43 Indv. /mL). For T6, the average number of gravid females was (57.1875 indv. /mL) which was (46 indv. /mL) at the first day of the experiment.

Comment [D12]: You did not mention in the method, how do you separate and count gravid females?

Comment [D13]: How can you compare among the treatments as initial stocking density of gravid females were big different (96, 43 and 46 indv/ml)

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Comment [D14]: How is T1, T2, T3 and T5 Where is T8 that didn't show in Figure 3

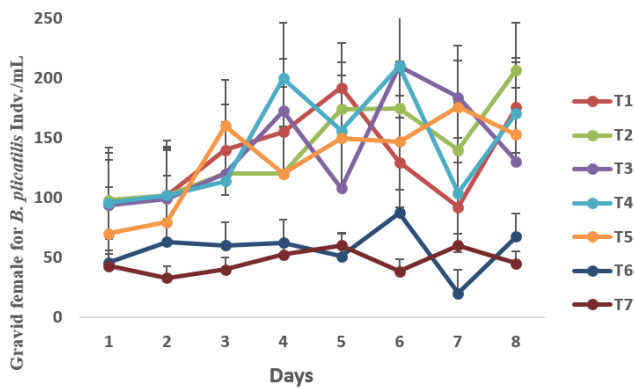


Figure 3: The gravid females of *B. plicatilis* (Indv./mL) during the experiment period.

Population Density and Egg ratio of *B. plicatilis*

Figure 4. Illustrated the relationship between the population density of the rotifer *B. plicatilis* and the egg ratio in the present study.

The population density of the studied rotifers increased exponentially with time for all the treatments and reached a noticeable level

after eight days (end of the experiment). The egg ratio decreased gradually over time during the different growth levels.

Comment [D15]: Did not mention in the method, how do you identify and count the egg from each treatment daily.

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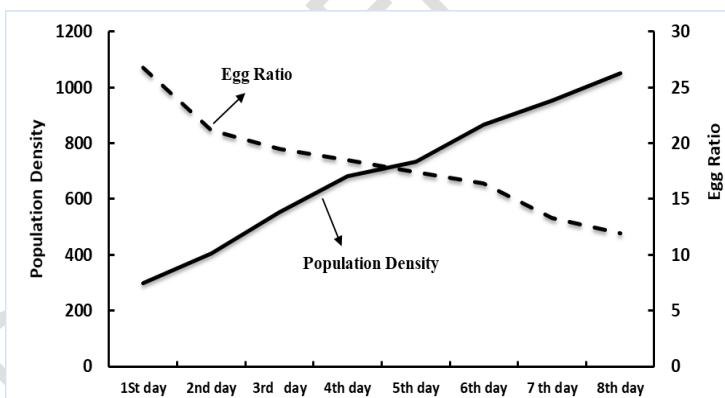


Figure 4: Schematic representation of changes in population density and egg ratio of *B. plicatilis* in relationship to time in the mean diet treatments.

4. DISCUSSION

Rotifers are cosmopolitan organisms as they are osmoconformers and can be adapted to salinities ranging from 1‰ to 97‰, however, the changes in salinity may cause shock for rotifers affects their production rate (Epp and Winston, 1977 and Walker, 1981). Increased

salinity may shorten the lifetime and decrease the fecundity of female and growth rate in some rotifer species (Serra *et al.*, 1994). where Oltra and Todoli (1997) found negative effect for decreasing salinity on growth rate of the marine rotifer, *Synchaeta cecilia valentine*. Numerous studies established the effect of salinity on growth rate of rotifer, *B. plicatilis* (Bosque *et al.*, 2001); where it can tolerate wide range of salinities (Bayly, 1972) but it has the highest reproductive rate at salinities ranging from 10- 20‰ (Miracle and Serra, 1989); They added that the ability of *B. plicatilis* to tolerate wide salinity range is due to its colony origin, where rotifers taken from the coastal marshes stand high salinities more than those taken from brackish water lagoons.

In the present study, the statistical analysis revealed that the eight diets showed different effects through the eight days of the experiment. The commercial diet T6 (S.parkle) ~~Caused~~caused gradual increase in rotifer density from the third; where Lind (2014) and Eryalcin, (2018) recorded the highest growth rate mean for S.parkle comparing with the other rotifer diets used in his study. Furthermore, Kostopoulou *et al.* (2012) found positive effect on the growth rate of the rotifer *B. plicatilis* with the dilution rate of S.parkle .

In contrast to the present and aforementioned findings, Qi *et al.* (2009) found an increased growth rate of rotifer cultures fed on *Nannochloropsis* algae than S.Parkle. More importantly, Reitan and Olsen (1994) found that S.parkle diet had promoted more bacteria than the algal diets; they found that yeast diet did not support growth rate of ~~rotifers-rotifers~~

where the Yeast *S. cerevisiae* was the less effective diet in the current study; this was confirmed by the results of Planas and Estevez (1989). In contrast, (Khatun *et al.*, 2014) reported that yeast is more effective than *Chlorella* powder as combined food with fresh *Chlorella*. Sarma *et al.* (2001) stated that the yeast was not appropriate for the rotifer cultivation but the mixture of yeast and fresh *Chlorella* could be more effective on the culture of the rotifer *B. calyciflorus* and better than the other mixtures (Ashraf *et al.*, 2010; Pena-Aguado *et al.*, 2005).

The egg ratio was determined in the present study in order to estimate the growth rate of *B. plicatilis*. Edmondson (1965) and Paloheimo (1974) reported that the egg ratio was used to evaluate the fecundity and the mortality rates for deriving the population growth rates. Ohman *et al.* (1996) and Razlutskiy (2000) had introduced a very useful bases for constant improvement in the use of the egg ratio method for zooplankton. There is usually an inverse, linear or curvilinear relationship between the density of population and the egg ratio. Duncan (1984), Sarma and Rao (1991) recorded that the planktonic rotifer populations continue to produce between 3 and 5 eggs per female per day at low population densities when there are plenty of resources.

5. CONCLUSION

The present study concluded that the commercial diet S.parkle was the most effective diet among the experimented diets in the current study, because it showed the highest growth rate compared with the other rotifer diets.

Comment [D16]: This discussion did not explain or support your result.

Comment [D17]: What did the effect of diets on the egg ratio?

Comment [D18]: Your result shown "The test revealed that the treatment pairs T1 and T3, T1 and T4, T1 and T5, T1 and T6, T1 and T7, T1 and T8, T2 and T5, T2 and T6, T2 and T7, T2 and T8, T3 and T6, T3 and T7, T3 and T8, T4 and T7, T4 and T8, T5 and T7, T5 and T8 had mean differences with significant value ($p < 0.05$). The rest pairs of treatments showed a significance level greater than (0.05) which means there were no statistically significant differences between them." Thus T4 and T6; T5 and T6; T6 and T7; T6 and T8 were not statistically significant. Therefore you can not concluded that T6 (S.parkle) is the most effective diets.

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