Original Research Article

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

Comment [OC1]: Can this title be rephrased? Must the salinity stress reflect in the title? Something like, Effect of different diets on the growth rate of *Brachionus plicatilis*

ABSTRACT

Aquaculture is important business some fish farms use underground water that may has high salinity. This study evaluated the effect of eight 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 with 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.

Comment [OC2]: The introduction given here is very weak and not showcasing the subject matter

Comment [OC3]: For what period of time did you subject the rotifers to these diets?

Comment [OC4]: All scientific names should be italicised for each treatment

Comment [OC5]: Did you subject the obtained data to any form of statistical test?

Statistical tools should be mentioned in the abstract!

Comment [OC6]: Population growth is the only result presented here, what of other results such as the relationship between egg and population?

Comment [OC7]: The statement here is not clear.

Comment [OC8]: Is there any form of recommendation from this study?

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

Comment [OC9]: Can these be arranged in alphabetical order?

1. INTRODUCTION

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

Comment [OC10]: Crops or species?

One of the vital difficulties faced production of fish fry in hatcheries is the lack of live food for the nutrition of early 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

Comment [OC11]: Do you mean challenges?

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.

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 litters initial volume containing 150 indv./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.

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

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

Comment [OC12]: Not listed under reference section

Comment [OC13]: The three references cited here are not listed under reference section.

Comment [OC14]: Beyond availability, is baker's yeast cost effective for the farmers?

Comment [OC15]: Really? Can other microalgae be more epensive than the baker's yeast?

Comment [OC16]: Since this live food (*N. sp*) is widely used, why do we need to research on this again?

Comment [OC17]: Not listed

Comment [OC18]: Is stocking density part of the objectives of this work?

Comment [OC19]: This looks confusing. Were the rotifers cultured at different salinity level? Reconcile between this objective and your broad topic.

Comment [OC20]: How were they transported from here to the study area? Under what condition?

Comment [OC21]: Were they randomized? Or how were they didivided to eliminate bias?

Comment [OC22]: 0-9 should be written in words and numbers greater than 9 can be written in figure.

Comment [OC23]: Give the model of the aerator used.

Comment [OC24]: Daily for how many days?

Comment [OC25]: Model number???

Comment [OC26]: There is need to justify the reasons for the selected feeds and not other species in the market.

Comment [OC27]: Is Selco the genus name of this alagae or S.?

Comment [OC28]: Why is N. Oculata considerd as control? And not S. Parkle? Give reasons for every action taken.

Comment [OC29]: What is the basis for these combination? In what ratio were they combined? Do we have the same cost implication for all these treatments?

Comment [OC30]: Why 8days and not more? Need to justify all action taken.

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

* Each one mL contains 150 indv. /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-Damhoghy *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:

$$R^r = \frac{(IN_t - IN_0)}{t}$$

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 ± 2.0°C and 40 ± 1.0 ppt, respectively.

Comment [OC32]: What mixing ratio did you use here?

Comment [OC31]: Reason for the asterik here?

Comment [OC33]: Why 0.1gm/l extra compared to the first three treatments?

Comment [OC34]: Not conspicous enough and not saying anything like notes or something!

Comment [OC35]: Why the broad name again? Consistency in he use of terms, rotifers.

Comment [OC36]: Years here should be in ascending order, 2014, 2015 2016 etc

Comment [OC37]: During?

Comment [OC38]: Or Bayly 1972?

Comment [OC39]: The fonts are not similar, be consistent.

Comment [OC40]: IN_0 described in the formula is different from N_0 defined here, please clarify.

Comment [OC41]: How did you test the homogeneity of data? Please state clearly. Some descriptive statistical tools used in this study were not mentioned.

Comment [OC42]: Do you mean average values for the measured water quality parameters?

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 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 on the ninth day was (935 Indv./mL), while the lowest average value recorded on 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.*

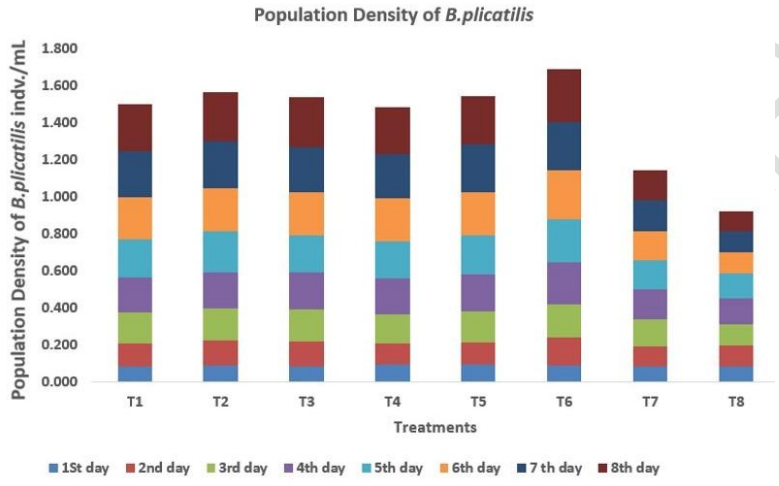

Figure1: Effect of the eight experimental diets during the experiment period.

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.

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.

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

Comment [OC43]: It is very important to italicise all the scientific names in the entire write-up.

Comment [OC44]: Experiment was carried out for eight days, why reporting the ninth day result?

Comment [OC45]: Confused already, what is the difference between this value here and 818ind/mL reported for T6???? and 265 indv as against 355 reported earlier as the least.

Comment [OC46]: Re-present the result in this statement for easier comprehension.

Comment [OC47]: Figure 1 revealed that T6 was the most .....

Comment [OC48]: The title «population density of B. plicatilis» should be removed from the figure.

Comment [OC49]: Include the standard error bars.

Comment [OC50]: Rephrase the title of this figure. E.g. Effect of eight experimental diet on what?

Comment [OC51]: You can't conclude within result section. Rephrase this statement or rather move to conclusion section.

Comment [OC52]: This is a new paragraph, what leads to the fact that? Not clear.

Comment [OC53]: This is statisitics in result section again.

Comment [OC54]: This is confusing. Must the pairs be listed? And why?

Comment [OC55]: The font here is different.

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).

Comment [OC56]: This does not tally with p=0.019 in the table.

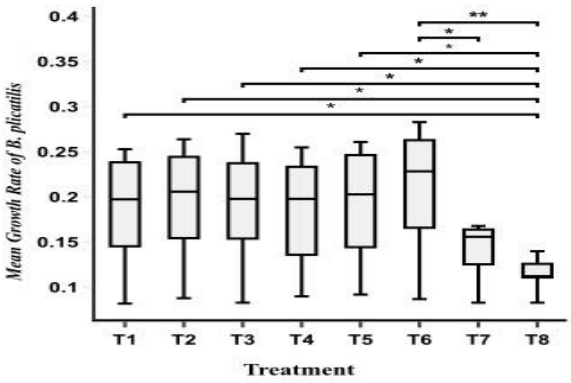

Figure 2: Boxplot provides a pairwise comparison between the experimental treatments (Duncan's multiple range test), the number of asterisks depends upon the *p*-value of particular pair of samples; * for $p < 0.05$ and ** for $p < 0.01$.

Comment [OC57]: Is this a title for figure 2 or an explanation of the figure?

Gravid females of *B. plicatilis*

Comment [OC58]: This is strange, the materials and methods did not say anything about gravid rotifers.

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 [OC59]: Not all values should be within the bracket. So recast and place the values appropriately.

How did you discern the gravid nature of the rotifers? Not mentioned in materials and methods. This part of your result was also not mentioned in the abstract section.

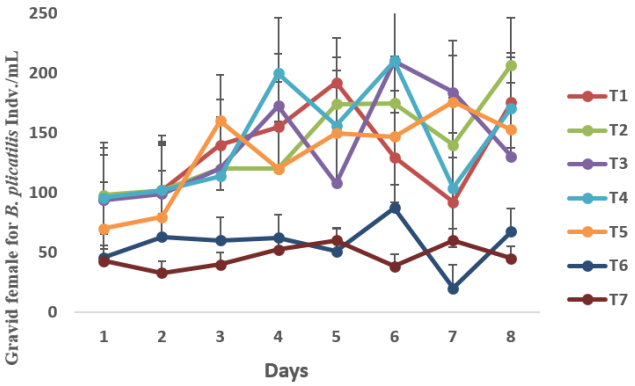

Figure 3: The gravid females of *B. plicatilis* (Indv. /mL) during the experiment period.

Population Density and Egg ratio of *B. plicatilis*

Figure 4. 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.

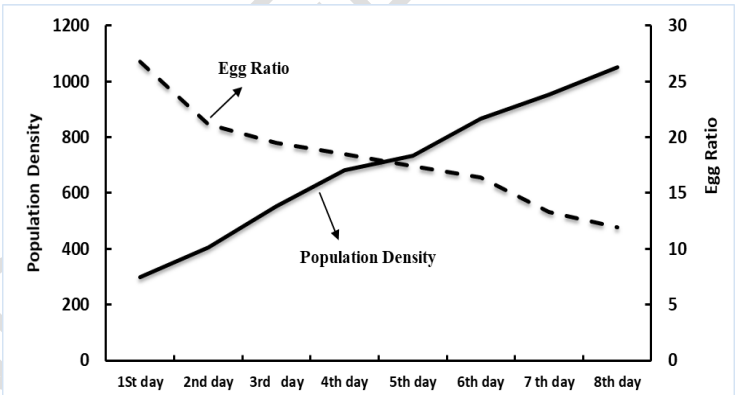

Figure 4: Schematic representation of changes in population density and egg ratio of *B. plicatilis* in relationship to time in the mean diet treatments.

4. DISCUSSION

Comment [OC60]: This was not also mentioned in materials and methods, and it is necessary for replication of experiment.

Comment [OC61]: A positive or negative effect of 40ppt should be reported.

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 origen, 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 a 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 rotefers 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 Razlutskij (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 [OC62]: Nothing was reported on the effect of 40ppt on the lifetime and fecundity in materials and methods and results. So why discussing things not reported?

Comment [OC63]: Is this part of the scientific name?

Comment [OC64]: Who are the they? Re-phrase

Comment [OC65]: Or origin?

Comment [OC66]: Or result?????

Comment [OC67]: Third what? Do you mean third day?

Comment [OC68]: Are these references corroborating or otherwise your own results? Results of this study should be discussed in line with other peoples' findings.

Comment [OC69]: State the findings of this work first before dining if its in tandem or otherwise to others.

Comment [OC70]: Only the year should appear in the bracket here.

Comment [OC71]: State the relationship oobserved in this study in comparism to reported findings.

Comment [OC72]: Is there any recommendation from the study?

REFERENCES

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Comment [OC73]: Or 2014?

Comment [OC74]: Not cited within the work.

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