

## Laboratory culture of *Brachionus plicatilis* in different salinity concentrations

Saba Abbas Kadhim

Adel k.Jassim

Naeem S. Hammadi

University of Basrah, College of Agriculture , Department of Fisheries and Marine Resources

E-mail: [adel.jassim@uobasrah.edu.iq](mailto:adel.jassim@uobasrah.edu.iq)

Received: 8 January 2023

Accepted: 12 March 2023

### Abstract

*Brachionus plicatilis* was cultured for ten days in the Marine Biology Department/Marine Science Center Basra at a temperature of 21 °C and with different salinity concentrations of 2, 4, and 10 ppt, and were fed with organic matter (cow waste) at a concentration of 3 g /l with continuous ventilation and lighting. The highest density was recorded at a salinity (10 ppt) (211,000 individuals/l), and the lowest density at a salinity (2 ppt) (30000 individuals / l). The growth rate( K )ranged from 1.03 - 1.22, and the reproductive rate ranged from 0.96 - 1.15 days for the same saline concentrations above (while the lowest value for doubling time D was 0.56 and the highest was 0.66 at 10 and 2ppt salinity concentrations, respectively). These are considered euryhaline rotifers, and the statistical analysis results showed significant differences between treatments at a significant level ( $P \leq 0.05$ ). The importance of this study lies in understanding the effect of salt on population density and determining the best salt concentration for the growth of the aforementioned rotifers.

Keywords: *Brachionus plicatilis*: Rotifers; Salinity; culture

### Introduction

Rotatoria is a group of relatively small, microscopic aquatic invertebrates comprising more than 2,000 identified species of unbranched, laterally symmetrical, and pseudo-coelomous animals (Segers, 2004). Although most rotifer species are freshwater, some species may be found in brackish water (estuaries) and marine environments (Fradkin, 2001). *Brachionus* is an indispensable zooplankton species in aquaculture systems and has been used as a primary live food for marine fish larvae since the 1960s. In

addition, this group is suitable for studying different aspects of ecology. Most farmers and breeders aim to conserve fingerling fish, and the genus *Brachionus* is an important food source (Francis, 2006).

The genus *Brachionus* is cultured using diets rich in essential nutrients to support the health status of fish larvae, increase their growth rates, and reduce the rate of mortality. (James & Abu-Rezeq, 1988). The possibility of using it as a live food is suitable for the larval stages of aquatic life

(Dhert 1996). (Abbas 2021) recorded the prevalence of the genus *Brachionus* in salinities ranging from 1.8-40 ppt.

The current experiment was conducted to study salinity tolerance at different salinity concentrations.

## Materials and methods

### Culture of *B. plicatilis*

Samples were collected from ponds near Basra University in Al-Tubah and Al-Nakhilah, where a bucket was collected several times. The collected water was then passed through several sieves, the first of which was the size of the holes (53  $\mu$ m) for zooplankton. It was adapted to a temperature of 21°C, and small individuals were collected (Ghazi, 2005), where 10 individuals were added to each tank of 5 liters of chlorine-free water at three salt concentrations, namely PPT 2, 4, and 10, and the amount of organic matter (cow dung) was fixed at 3 g/L. Other laboratory environmental factors were confirmed, as in Table No. (1), where the temperature was controlled using a thermostat and thermometer daily during the culture period, which lasted for ten days, after which a sample was taken from each tank and counted using a counting slide and slide-counting compound microscope under a force of 10X40X. The process is repeated (3 times, and the average is taken) for each basin, then the number was calculated to obtain the final densities of each fry and according to the concentrations mentioned in the study, and the result was expressed in individuals/liter. Growth indicators were calculated according to the equations set by Scott and Baynes (1978), and the growth rate (k) and doubling time (D) were measured using the following equation:

$$K = \frac{\ln N_t - \ln N_0}{T}$$

$$D = \frac{\ln 2}{K}$$

whereas:

$N_t$  = final number

$N_0$  = prime number

T = time

= Log e2 fixed

K = growth rate

And calculate the reproductive rate (G) Reproductive Rate/day as described before (Euteneuer et al, 1984)

$$G = (1/T) (\ln N_t / N_0)$$

whereas:

T = time

N = final number (egg-bearing and non-egg-bearing)

$N_0$  = the prime number

## Results and discussion

### 1:- Environmental factors

Table No. (1) shows the basic environmental factors in which *B. plicatilis* was cultured at a temperature of 21°C, dissolved oxygen 7.4 mg/L, and pH 7.6.

### 2:- *B. plicatilis* . culture

Table No. (2) shows the total numbers obtained after culturing *B. plicatilis* at a fixed organic concentration of 3 g/L, different salinity concentrations, and stabilization of the rest of the environmental factors, where the lowest reached 30,000 individuals/l. The highest recorded was 211,000 individuals/l at saline concentrations of 2 and 10 p.p.t. (Fig. 1), and the density increased with increasing salt concentration. The growth rate K ranged from 1.03 - 1.22 (Fig. 2), and the reproductive rate ranged from 0.96 - 1.15 days (Fig. 4) for the same salinity concentrations above (while the lowest value for doubling time D was 0.56 and the highest was 0.66 at 10 and 2 p.p.t salt concentrations, respectively (Fig. 3). The results of the statistical analysis showed that there were significant differences between the treatments at the level of significance ( $P \leq 0.05$ ).

**Table 1: Laboratory Environmental Factors During Culture**

The environmental factor of pond water	value $\pm$ standard error
<b>Temperature C°</b>	21 $\pm$ 1
<b>pH</b>	7.6 $\pm$ 0.02
<b>Dissolved oxygen mg/L</b>	7.4 $\pm$ 0.02

Table 2. Total numbers, growth rates, doubling time, and reproductive rate obtained after culturing *B. plicatilis* in different organic and salinity concentrations for ten days.

Organic matter g/L	salinity concent rate ppt	Prime number individual/l	Final number individuals/l	Growth rate K	Doubling time D	Reproduction rate G
3	2	2	30 $\times$ 10 <sup>3</sup>	1.03	0.66	0.96
3	4	2	50 $\times$ 10 <sup>3</sup>	1.08	0.63	1.01
3	10	2	211 $\times$ 10 <sup>3</sup>	1.22	0.56	1.15

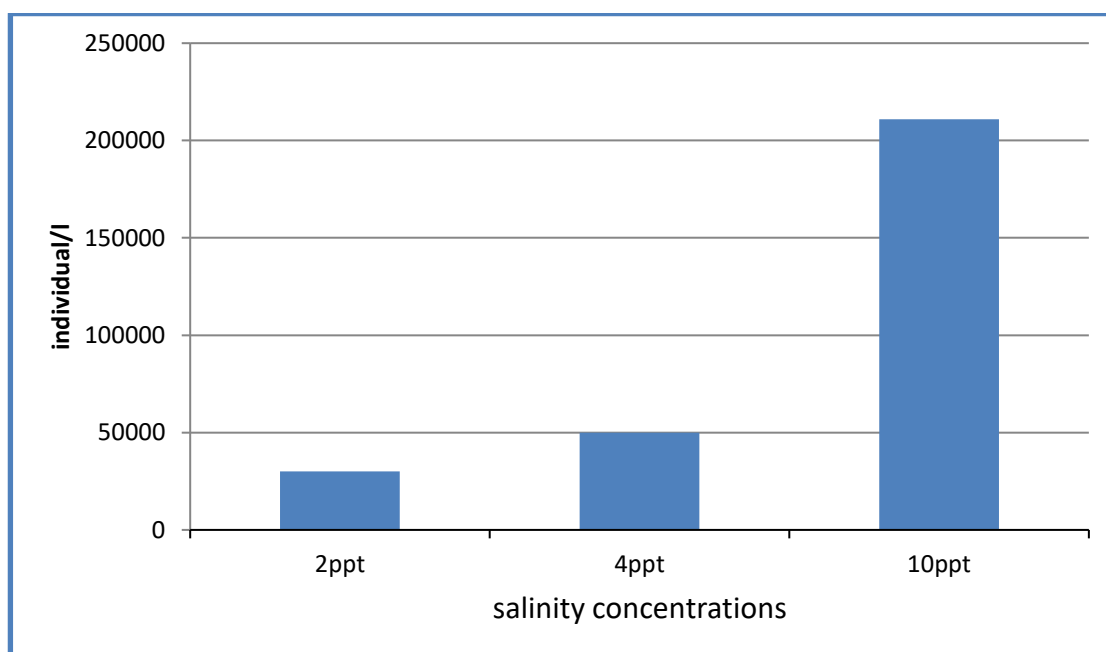


Figure 1: Total numbers obtained after culturing *B. plicatilis* at a constant organic concentration of 3 g/L and at different salinity concentrations for ten days.

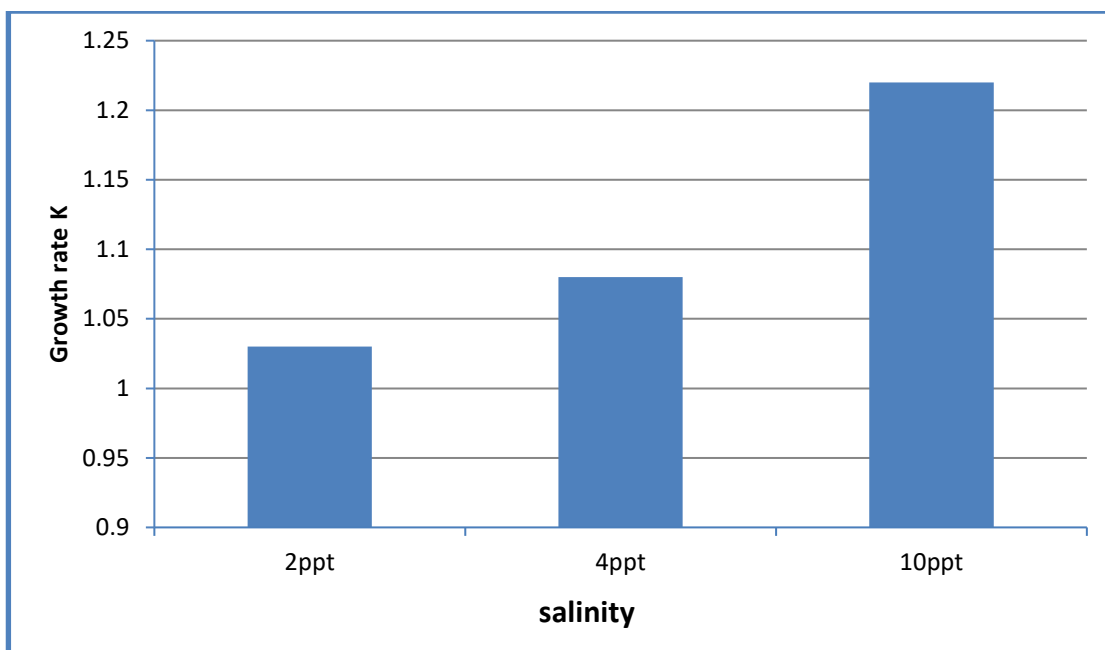


Figure 2: Growth rates of K obtained after culturing *B. plicatilis* at a constant organic concentration of 3 g/L and different salinity concentrations for ten days.

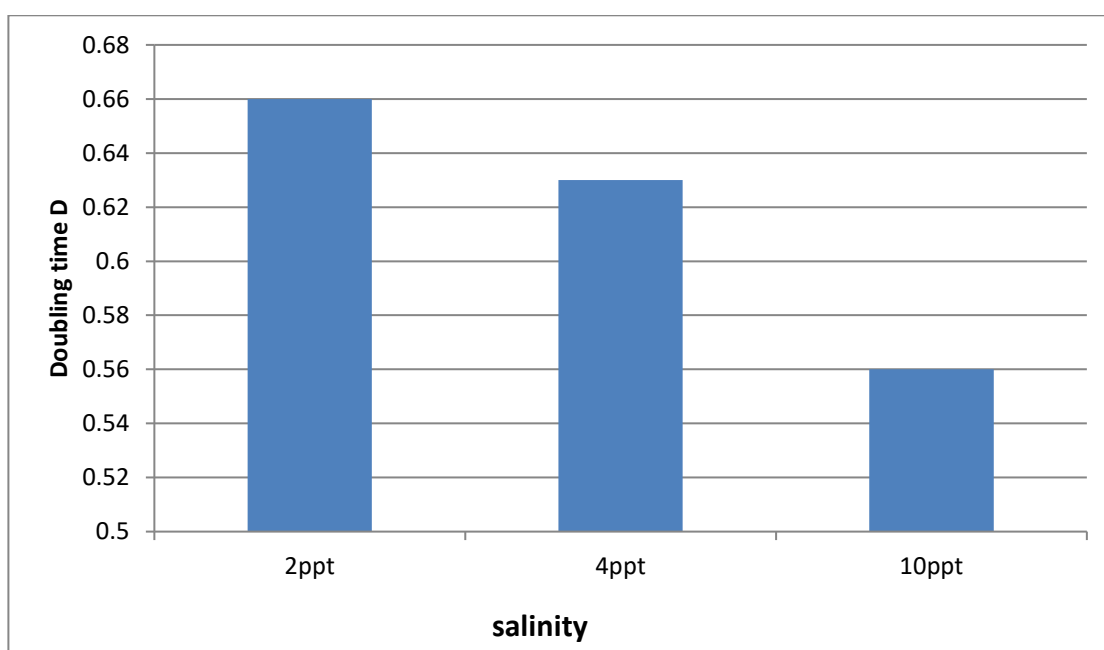


Figure 3: Doubling time D obtained after culturing *B. plicatilis* at a constant organic concentration of 3 g/L and at different salinity concentrations for ten days.

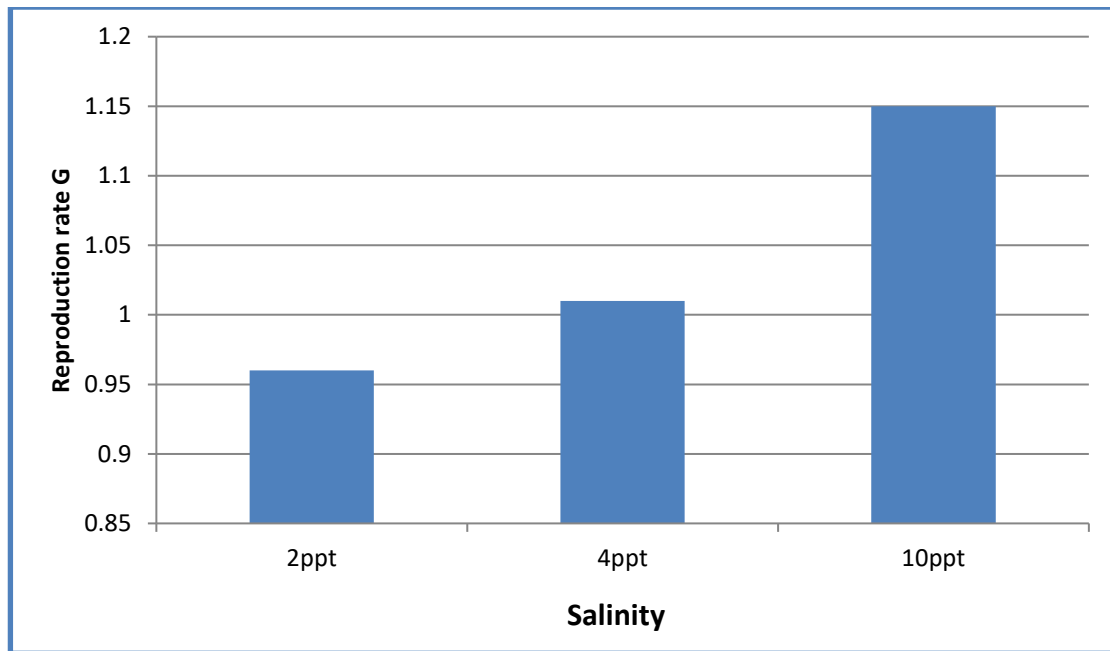


Figure 4: Reproduction rate G obtained after culturing *B. plicatilis* at a constant organic concentration of 3 g/L and at different salinity concentrations for ten days.

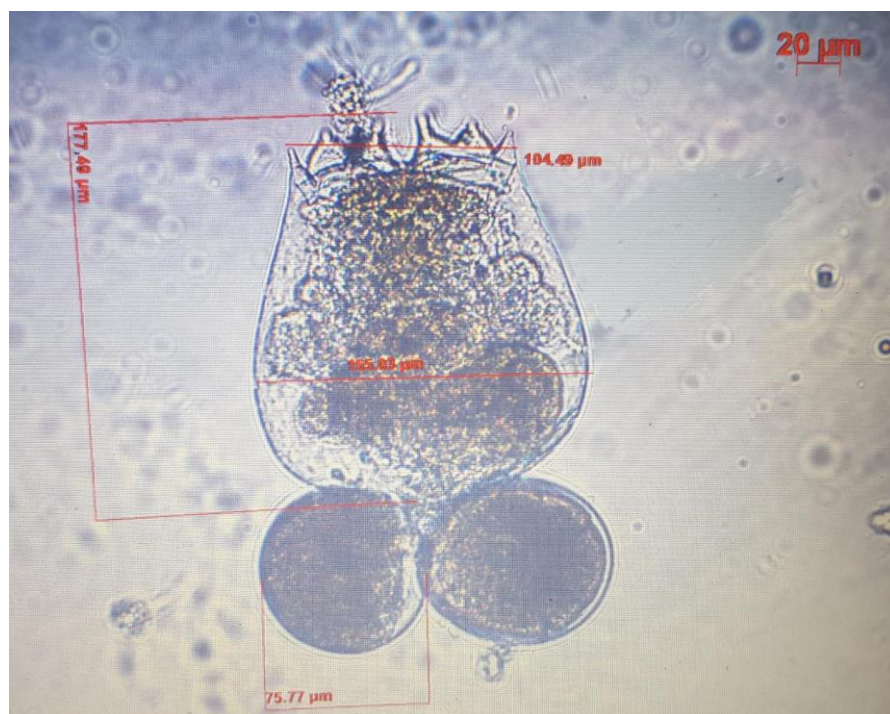


Figure5: small model (S) for type *B. plicatilis*

Temperature selection is important in rotifer culture; two types of *Brachionus plicatilis* are dependent on temperature: the first small type (S), which ranges from 80

to 250  $\mu\text{m}$  (Tamaru et al., 1995), and the second largest type (Tamaru et al., 1995), which reaches 400  $\mu\text{m}$ , is produced at low temperatures (Dhert, 1996). Therefore, the

choice of temperature in the current study and in the field was closest to the lowest temperatures to obtain the type with the target size (S), as shown in Figure 5; the highest length was 220  $\mu\text{m}$  and the width 115  $\mu\text{m}$  was recorded at a salinity of 10. In comparison, the lowest recorded length was 135  $\mu\text{m}$ , and the width was 98  $\mu\text{m}$ , which is consistent with the study by (Snell and Carrillo 1984).

This application is of particular importance in aquaculture to find pinwheels that fit the size of the mouth opening of the larvae that feed on them, because the rotifers of the large model in size do not match the size of the mouth opening of fish and crustacean larvae in the first stages. Salinity was chosen to suit the liquefaction water, which did not exceed 4p. p.t. At the time of the study, two degrees of salinity were chosen: the highest and the lowest, PPT(2,10), respectively. To determine the effect of these concentrations on the numerical densities that can be obtained, this type is characterized by high salt tolerance and can withstand concentrations ranging from 1 to 97 parts per thousand; however, the ideal range for this type is 35 parts per thousand (Ahmed and Ghazi, 2009; Lubzens, 1987). The cultured species *B. plicatilis* is widely used as food for fish and shrimp, especially for species that live in saltwater, where it is considered a marine rotifer, and many international studies have focused on its use as food (Watanabe et al., 1978; Lubzens, 1987; Lim et al., 2003). As for the salinity effect, the highest density was recorded in the current study at the highest salt concentration (10ppt), which is consistent with the study by Weber and Juanico (2004), which showed that increasing the values of salts extracted from the drainage water in the culture medium increased the density of living organisms.

### Conclusions

This type is considered to have high salinity tolerance, as it gives the highest

densities when salinity increases, which slightly affects its size.

### Acknowledgement

I would like to express my sincere gratitude to the University of Basrah, College of Agriculture, Department of Fisheries and Marine Resources for their invaluable support and guidance throughout my research.

### References

- Abbas, M. F. (2021). An ecological study of the Rotifera, south of the Shatt al-Arab River, Basra, Iraq. *Iraqi Journal of Aquaculture*, 18(2): 1-16.
- Ahmed, H.K., and Ghazi, A.H. (2009). A taxonomic and environmental study of the genus *Brachionus* in Al-Hammar marsh, South of Iraq. *Iraqi J. Aquacul.*, 6(2): 105 – 112.
- Arimoro, F. (2007). First feeding in the African catfish *Clarias anguillaris* fry in tanks with the freshwater rotifer *Brachionus calyciflorus* cultured in a continuous feedback mechanism in comparison with a mixed zooplankton diet. *JFish. & Aqua.Sci.*2(4):275-284.
- Hanoon, Kamal (2005). Cultivation of some types of plankton used as food for fish larvae and young. *Tishreen University Journal of Scientific Studies and Research*, 27(2): 35-50.
- Ghazi, Abdel-Hussein Hatem (2005). Using live food in rearing larvae of *Cyprinus carpio* and *Ctenopharygodon idella* fish larvae. Master's thesis, College of Agriculture, University of Basra, 89 pages.
- Gower, Haifa Jawad; Awad, Abd al-Husayn Habash; Abdel-Wahhab, Ismail (1986). *Practical Invertebrates*. College of Education, University of Basra, Ministry of Higher Education and

- Scientific Research, Dar al-Kutub for Printing and Publishing, 112 p..
- Hammadi, Naim Chand and Salman, Daoud Salman and Al-Issa, Saleh Abdel Qader (2012). Wheels of Nahr Shatt Al Arab Basra, Iraq. 258 p.
- Baboli ,J.M. and Hosseini, S. J.(2015) Effect of Light Intensities on Growth and Reproductive Of Rotifer *Brachionus Rotundiformis* .9(1):1-9.
- Chengalath, R. 1985. The Rotifera of the Canadian Arctic Sea ice ,with description of a new species. Can. J.Zool.63:2212-2218.
- Dhanapathi ,M.V.(1977). Student on the distribution of *Brachionus calyciflorus* in India.Arch. Hydrobiology Ergebn.Limnol.,8:226-229. Dhert, P. 1996. Manual on the production and use of live food for aquaculture. FAO Report. 295 pp.
- Dhert, P. 1996. Manual on the production and use of live food for aquaculture. FAO Report. 295 pp.
- Dhert, P., Rombaut, G., Suantika, G. and Sorgeloos, P. 2001. Advancement of rotifer culture and manipulation techniques in Europe. Aquaculture 200:129–146.
- Edmondson,W.T. (1959). Reproduction rates of plankton rotifers as related to food and temperature in nature. Ecol.Monogr.,35: 61-112.
- Euteneuer, S.,Lubzent,E. and Fishler,R(1984). A preliminary report on cold preservation of the rotifer *Brachionus plicatilis* (O. F. Müller ). In Rosenthal ,H .and sarig S.(eds),Research on Aquaculture, Europ.Mar.Soc.8:211-228.
- Fradkin,S.C. 2001. Rotifer distributions in the coastal waters of the northeast Pacific Ocean. Hydrobiologia ,446/447:173-177.
- Francis O. Arimoro (2006). Culture of the freshwater rotifer, *Brachionus calyciflorus*, and its application in fish larviculture technology. Afracan Journal of Biotechnology 5(7):536-541.
- James, C. M. and Abu-Rezeq, T. T. (1988). Effect of different cell density of *Chlorella capsulata* and a marine *Chlorella* sp. For feeding the *Brachionus plicatilis*. Aquaculture ,89:43-56.
- Lawrence, C.; Sanders, E. and Henry, E. 2012. Methods for Culturing Saltwater Rotifers (*Brachionus plicatilis*) for Rearing Larval Zebrafish. Zebrafish, 9(3): 140-146.
- Lubzens, L. 1987. Raising rotifer for use in aquaculture. Hydrobiology. 147:245-255.
- Schnack-Schiel, S.B.2003.The macrobiology of sea ice.In: Thomas D.N.& Dieck mann,G.S. (eds.) Sea Ice:An Introduction to its physics, Biology and Chemistry.) Blackwell Publishing, Inc., Malden, MA:Chapter 7.
- Scott, A.P. and Baynes, S.M. 1978. Effect of algal diet and temperature on the biochemical composition of the rotifer *Brachionus plicatilis*. Aquaculture. 14:247 – 260.
- Segers,H.(2004).Rotifera:Monogonota.In: Yule,C.M.& H.S.Yong(eds) Fresh water Invertebrates of the Malaysian Region.Academy of Sciences Malaysia and Monash University,Malaysia, Kuala Lumpur:112-126.
- Tamaru, C.S.; Cholik F.; Kuo, J.C.M. and FitzGerald, J.JR.(1995). Status of the culture of milkfish (*Chanos chanus*), striped mullet (*Mugil cephalus*) and grouper (*Epinephelus* sp.) Reviews in Fisheries Science 3(3):249-273.
- Wallace, R. L. . Snell, T.W. Ricci,C., and Nogrady,T. ( 2006). Rotifera: Volume 1

- Biology, Ecology, and Systematics. Guides to the Identification of the Microinvertebrates of the Continental Waters of the World 23 (Dumont, h.j., ed.). Kenobi Productions, Ghent, and Backhuys Publishers, Leiden.
- Wallace, R. L. and T.W. Snell (2001). Rotifera. In Ecology and Classification of North American Freshwater Invertebrates, 2nd edition. (Thorpe, J. & Covich, eds.) Academic Press, NY.: 195-254.
- Weatanabe, T.; Kitajima, C.; Arakawa, T.; Fukusho, K. and Fujita, S. (1978). Nutritional quality of rotifer *Brachionus plicatilis* as a live feed from the view point to essential fatty acids for fish. Bull. Jap. Soc. Sci. Fisheries, 44:1109-1114.
- Werner, I. and Auel, H. 2004. Environmental conditions and overwintering strategies of planktonic metazoans in and below coastal fast ice in the Gulf of Finland Baltic Sea. Sarsia., 89:102-106.
- Wetzel, R.G. (2002). Limnology, lake and river ecosystems. 3rd ed. Academic Press, An Elsevier Science Imprint, San Francisco, New York, London.
- Yoshimatsu, T.; Higuchi, T.; Hamasaki, Y.; Tanaka, K. (2008). Preliminary trials on the effect of lighting on the population growth of the rotifer *Brachionus plicatilis*. [Japan Agricultural Research Quarterly- JARQ- ER 42\(2\)](#):

### الاستزراع المختبري للدولابي *Brachionus plicatilis* في تراكيز ملحية مختلفة

نعيم شند حمادي

عادل قاسم جاسم

صبا عباس كاظم

المستخلص

تمت تربية *Brachionus plicatilis* لمدة عشرة أيام في قسم الأحياء البحرية / مركز العلوم البحرية بالبصرة عند درجة حرارة 21 درجة مئوية وبتراكيز ملوحة مختلفة 2 و 4 و 10 جزء من المليون وتم تغذية المستزرعة بالمواد العضوية (نفايات الأبقار) بتركيز 3 غم / لتر مع تهوية وإضاءة مستمرين. سجلت أعلى كثافة عند الملوحة (10 جزء الف) (211.000 فرد / لتر) وأقل كثافة عند الملوحة (2 جزء بالف) (30000 فرد / لتر). تراوح معدل النمو (K) من 1.03 - 1.22 ، وتراوح معدل التكاثر من 0.96 - 1.15 يوماً لنفس تركيزات المحلول الملحي أعلاه (بينما كانت أقل قيمة لمضاعفة الوقت D 0.56 وأعلى 0.66 بتركيزات ملوحة 10 و 2 جزء من المليون. يعتبر هذا النوع من الروتيفيرات الملحية وقد أظهرت نتائج التحليل الإحصائي وجود فروق ذات دلالة إحصائية بين المعاملات عند مستوى معنوي ( $P \leq 0.05$ ). تكمن أهمية الدراسة في معرفة تأثير الملح على الكثافة السكانية وتحديد أفضل تركيز ملوحة لنمو الروتيفيرات المذكورة

كلمات مفتاحية: الدولابيات: الملوحة : تحمل زراعة.