



International Journal of Fisheries and Aquatic Studies

E-ISSN: 2347-5129

P-ISSN: 2394-0506

(ICV-Poland) Impact Value: 5.62

(GIF) Impact Factor: 0.549

IJFAS 2017; 5(1): 13-17

© 2017 IJFAS

www.fisheriesjournal.com

Received: 04-11-2016

Accepted: 05-12-2016

Matus LN

Universidad Autónoma Metropolitana
Xochimilco, Depto. El Hombre y su
Ambiente, Live Food Production
Laboratory, Biology degree, Secondary
Production Module, Calz. del Hueso No.
1100, Col. Villa Quietud, Ciudad de México.

Rodríguez RV

Universidad Autónoma Metropolitana
Xochimilco, Depto. El Hombre y su
Ambiente, Live Food Production
Laboratory, Biology degree, Secondary
Production Module, Calz. del Hueso No.
1100, Col. Villa Quietud, Ciudad de México.

Jiménez LA

Universidad Autónoma Metropolitana
Xochimilco, Depto. El Hombre y su
Ambiente, Live Food Production
Laboratory, Biology degree, Secondary
Production Module, Calz. del Hueso No.
1100, Col. Villa Quietud, Ciudad de México.

Castro MJ

Universidad Autónoma Metropolitana
Xochimilco, Depto. El Hombre y su
Ambiente, Live Food Production
Laboratory, Biology degree, Secondary
Production Module, Calz. del Hueso No.
1100, Col. Villa Quietud, Ciudad de México.

Ocampo CJA

Universidad Autónoma Metropolitana
Xochimilco, Depto. El Hombre y su
Ambiente, Live Food Production
Laboratory, Biology degree, Secondary
Production Module, Calz. del Hueso No.
1100, Col. Villa Quietud, Ciudad de México.

Castro MG

Universidad Autónoma Metropolitana
Xochimilco, Depto. El Hombre y su
Ambiente, Live Food Production
Laboratory, Biology degree, Secondary
Production Module, Calz. del Hueso No.
1100, Col. Villa Quietud, Ciudad de México.

Dávila F

Universidad Autónoma Metropolitana
Xochimilco, Depto. El Hombre y su
Ambiente, Live Food Production
Laboratory, Biology degree, Secondary
Production Module, Calz. del Hueso No.
1100, Col. Villa Quietud, Ciudad de México.

Castro CAE

Universidad Autónoma Metropolitana
Xochimilco, Depto. El Hombre y su
Ambiente, Live Food Production
Laboratory, Biology degree, Secondary
Production Module, Calz. del Hueso No.
1100, Col. Villa Quietud, Ciudad de México.

Correspondence

Castro MJ

Universidad Autónoma Metropolitana
Xochimilco, Depto. El Hombre y su
Ambiente, Live Food Production
Laboratory, Biology degree, Secondary
Production Module, Calz. del Hueso No.
1100, Col. Villa Quietud, Ciudad de México.

Population density comparison of *Brachionus angularis* (Gosse, 1851) cultured in laboratory at 19°, 21° and 25 °C, fed with bacteria produced in Biofloc system

Matus LN, Rodríguez RV, Jiménez LA, Castro MJ, Ocampo CJA, Castro
MG, Dávila F and Castro CAE

Abstract

The use of heterotrophic bacteria as only food in rotifers diet to make massive production beakers has been poorly studied, that is why this study used *B. angularis* cultivated by triplicate in 20 L plastic beakers at temperatures of 19°, 21° and 25 °C, with continuous light and aeration (24 hours), during 60 days. Bacteria was obtained from screened liquid (20 µm) grown in tilapias Biofloc system. Every third day a sample of 500 mL was taken from each beaker and population density was determined. Started experiment, after third day, at 19 °C total organisms died. The maximum density was 18.078 ± 187 (21 °C) and 15.972 ± 157 (25 °C). The reproduction rates were: $R_0 = 58.16$ and 51.49 org.female⁻¹; $T_c = 40.50$ and 40.7 days respectively and both have $r = 0.10$. The total density did not exceed 2 org.mL⁻¹. However, heterotrophic bacteria were used as a food to maintain low density rotifer culture or in mixed diets with microalgae.

Keywords: *B. angularis*, bacteria, culture, temperature, reproduction rates

1. Introduction

Aquaculture is an activity that includes several practices and a wide range of species, systems and production techniques. In aquaculture, live food consisting principally plankton group (phytoplankton and zooplankton), which constitutes the basic unit of production of organic material in aquatic ecosystems. Among various species in zooplankton, rotifers of genus *Brachionus* sp. have been regularly used as live food for other organisms, they differ in lorica length: 130-340 microns (239 microns average) for L type and 100- 210 microns (160 microns average) for type S. There are also differences in weight, shape of the occipital spine and optimal growth temperatures (L type has a wider temperature range, while S type has greater resistance at high temperatures).

Brachionus angularis, is one of smaller rotifer species (84 ± 4.9 to 127.8 ± 5.9) [1, 2] has two types of reproduction, by parthenogenesis and sexual. They are nonselective filter feeders, consume microalgae, bacteria, yeast and protozoa. To optimize rotifer culture production, it has been used different microalgae like *Nannochloropsis* sp., *Tetraselmis* sp., *Chlorella vulgaris*, *Isochrysis* sp., *Haematococcus pluvialis* and *Sphaerocystis* sp. which have high nutritional quality [3]. During rotifer production, it is required large amounts of living microalgae as food, but their high costs, contamination risk and temporal variations in their nutritional value, are problems for any aquaculture operation system [4,5].

Also, nutritional supplements are used to replace live microalgae, such as yeasts and bacteria, which can be a potentially effective alternative to optimize live food production and supply continuously demand rotifers in production systems [5], but with one disadvantage that 60% of food supplied is not used by organisms, causing accumulation of different compounds like phosphorus, carbon and nitrogen, among others, remaining in water column as suspended matter, as dissolved chemicals or expelled from system by gasification, or replacement of cultured water, contaminating other nearby water bodies and soils, causing economic losses to producers [6, 7].

The cost-benefit in rotifer production was supported using a cheap source of food, therefore baker's yeast can be used as diet but must be considered as a partial or complete substitute for microalgae diet in these filter feeder species, because microalgae cannot contain some

Essential fatty acids or they are in low content [8]. For this reason, we proposed a bacteria diet for *B. angularis* production, using rich bacteria water source, after three weeks cultured in tilapia Biofloc system at different temperatures (19°, 21° and 25 °C). Bacteria source acts as a nutrient trap retention in ponds, which lower maintenance costs and can be used as supplement food to produce massive rotifer cultures, improving rates of food utilization [7].

2. Material and Methods

Rotifers population used in this experiment were obtained from Live Food Production Laboratory from El Hombre y su Ambiente Department of Universidad Autónoma Metropolitana Xochimilco Unit, located at 19°18'11'' N; 99°06'07'' W.

2.1 Experimental design

Rotifers culture was made in 20 L plastic beakers, fulfilled with 15 L of tap water (Fig.1), making three replicas for each experimental temperature (19°, 21° and 25 °C). The culture system had constant white light (40 w) and aeration. The beakers were inoculated with 309 org.L-1 and fed every third day with 2 L of bacteria source obtained by tamized water (20 µm) from tilapia Biofloc system.

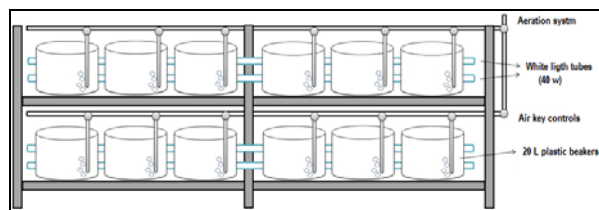


Fig 1: *B. angularis* culture system used in laboratory at different experimental temperatures (19°, 21° y 25 °C).

2.2 Feeding

To obtain bacteria to feed *B. angularis* experiments, were installed four beakers of 200 L capacity with 160 L of tap water with 35 tilapias in juvenile stage, fed with trout pellet (60% protein) and enriched with molasses as carbohydrates source to produce Biofloc. This system was installed three weeks before beginning rotifers culture to obtain heterotrophic bacteria.

From each plastic beaker, it was obtained 3 L culture media and pass through a nylon mesh of 20 µm to detain zooplankton and organic matter and obtain only bacteria liquid media. Then, 2 L from each rotifer experimental culture media was extracted and replaced with 2 L of food bacteria media.

2.3 Density samples

Each third day (during 60 days), from each experimental beaker a 500 mL simple was taken and pass through a sieve (20µm) to obtain rotifers and concentrated in 50 mL beaker. Three 0.1 mL subsamples were taken and fixed with Lugol solution (5%). The rotifers were counted using a stereoscopic microscope. Data were extrapolated to 20 L.

2.4 Processing data

Population density values were introduced in Excel 2010 base data to obtain mean values (±S.D.) and tendency growth

curves.

Density values were introduced in Life Table Program (Excel 2010) to obtain reproductive parameters:

Reproduction rate: $R_o = \sum l_x * m_x$

Where:

\sum = summatory

l_x = survival proportion from each phase

m_x = produced organisms from each survival organism from each phase

Growth intrinsic rate: $r = \log_e R_o / T_c$

Where:

$\log_e R_o$ = reproduction rate natural logarithm

T_c = Cohort generational time

Cohort generational time: $T_c = \sum x * l_x * m_x / R_o$

Where:

\sum = summatory

l_x = survival from each phase

m_x = produced organisms from each phase

R_o = Reproduction rate

2.5 Statistical

With population density mean values obtained from each experimental temperature, significant differences ($P < 0.05$) were determined by ANOVA analysis using Systat 13.0 statistical program and multiple mean values comparison (Tukey's test).

3. Results

Rotifers cultivated at 19 °C died at third culture day. Table 1 and Fig. 2 shows mean population density values every third day. Experimental rotifer cultures at 21°±2 °C reach densities of 18,078 ±187 org. 20L-1, whereas cultured experiments at 25 °C±2 °C obtained values of 15,972±157 org. 20L-1. In both experimental tests temperatures, growth tendency shows polynomial second grade curves. ANOVA test showed significant differences between them ($P < 0.001$).

Table 1: Population density mean values (±S.D.) of *B. angularis* cultivated in three experimental temperatures.

Sample day	Culture experimental temperatures		
	19 °C	21 °C	25 °C
0	309±21	309±23	309±22
3	---	139±19	134±14
6		81±13	61±12
9		133±13	89±7
12		297±27	218±18
15		573±35	447±22
18		960±60	777±23
21		1,458±14	1,208±28
24		2,068±20	1,739±17
27		2,790±27	2,371±31
30		3,622±23	3,104±30
33		4,566±66	3,938±39
36		5,622±22	4,872±45
39		6,789±78	5,907±90
42		8,067±80	7,043±74
45		9,457±45	8,279±89
48		10,959±159	9,616±91
51		12,571±125	11,054±110
54		14,295±124	12,592±592
57		16,131±311	14,232±234
60		18,078±187	15,972±157

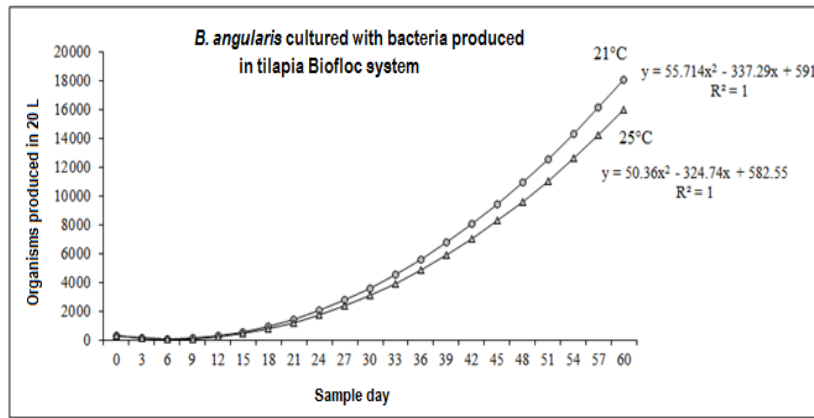


Fig 2: Population growth tendency curves of *B. angularis* produced in laboratory at two temperatures and fed with bacteria produced in tilapia Biofloc system.

Table 2 show the mean values in Life Table program, which observed differences organisms produced per organism, at 21 °C each rotifer produced 58 new organisms and at 25 °C, 51

new ones. Cohort generational time were 40 days and growth intrinsic rate were 0.10 in both temperatures

Table 2: Reproduction rates data of *B. angularis* obtained at two experimental temperatures in laboratory cultures.

Experimental culture temperature	Reproduction rate $\sum l_x * m_x$ Ro	Cohort generational time $\sum x * l_x * m_x / Ro$ Tc	Growth intrinsic rate $\log_e Ro / Tc$ r
21 °C	58.16	40.50	0.10
25 °C	51.49	40.70	0.10

4. Discussion

Temperature is an important factor for growth and reproduction for most organisms and rotifer group is not an exception. *B. angularis* showed a higher growth rate at 20-25 °C [9, 10], but another study [11], mentioned that it is better between 18-25 °C for their culture. In *B. calyciflorus* it has been worked in temperatures of 18°, 23° and 28 °C [12], but these authors agree that any rotifer specie behave in culture temperature and any rotifer population decrease their life expectancy when temperature increase up their optimal range, and their reproduction decrease when culture temperature is <20 °C, similar we found in this study with *B. angularis*. The density decrease or their total mortality at first culture days correspond with other authors [1], which worked with same rotifer specie and found that in 20 °C culture medium, fecundity diminished and need more time to begin reproduction.

The importance of increasing temperature in *B. angularis* culture medium was reflected in body size [13], whereas, other studies mentioned that temperature factor was the main factor that control rotifer population dynamic and specially for *B. angularis* [14]. This was observed in Jinhu Lake during summer and autumn stations where rotifers showed better fertility at 18 °C and better reproduction, growth and birth rate at 20 °C. This differs with our findings in 19 °C, where all rotifer population died at third culture day. These authors [14], mentioned that at 25 °C temperature the new born rotifers of *B. angularis* showed a better growth rate and reach adult stage in fewer days, aversely with our study where best temperature was 21 °C. It is important to considered species or population origin of *B. angularis* because everyone present a different behavior depending their geographical biotope [15]. One of the most important factors for rotifer culture was the food, but also their concentration which was needed to be maintained at 0.4 mg.CL-1 to 5.0 mg.CL-1 range to obtain growth rates (r) of 1.57 ±0.07 [16]. These authors work with

Nannochloropsis oculata microalgae in *B. plicatilis*, *B. nevada* and *B. cayman* cultures, finding negative growth rates when concentration is below 4.0 mg.CL-1 and when food has high concentration, reproduction rate increase. It was observed in many rotifer species that bigger organisms increase their energetic reservoirs and diminished their metabolic rates. When food concentration was low most of rotifer species presents similar growth rates.

When *B. angularis* is cultured with live and dry *Chlorella* sp. enriched with yeast [17], population density reach 60, 50 and 30 org.mL-1, upper values obtained for this study with bacteria produced by Biofloc system, which only obtained 15 to 18 org.mL-1. These authors mentioned that it is important when you are going to use a new experimental diet, an acclimation period with the experimental diet, but is necessary to mix two different diets, for example microalgae and bacteria or live and dried diet to complement their nutritional value.

Studies with *B. angularis* [15], fed with *Scenedesmus obliquus* founded densities of 2000 org.mL-1, an r = 0.11, very similar with this study (r = 0.10), a Tc = 25-44 days, also similar with this study with 40 days and Ro = 13-16, lower values with respect to this study with 51-58 org.hembra-1. These authors mentioned that Ro values, that was specific for each specie or population. It also mentioned, when food was poor in nutrient content or concentration, birth of myctic females decrease and can reach values of cero. It was important to considered that mechanism that this organism must accepted some food particles type, consisted in their size, which need to be <20. That's why this experiment sieve Biofloc liquid from a mesh of 20 µm.

It is important to considered environmental conditions of geographical places where rotifer samples were taken, because same conditions need to be placed in laboratory condition to make the experiments [1]. Also, when temperature increased in culture medium, the water began to deteriorated

and affect growth and reproduction rotifer populations. These conditions can be observed in 25 °C cultures in this study, in which it was observed a higher Ro at 21 °C (58 org. female-1). This is different than what was obtained by other authors [12], which worked with *B. calyciflorus* and mentioned that r value was determined for rotifer genetic and influenced principally for density population. The origin of rotifer strain is very important, because this value can modify Ro value for each cultured population. Each rotifer specie or population can be used different reproductive strategies to respond to environmental and culture conditions like light, food and density [18].

Researchers that worked with *B. angularis* [14] founded r values of 0.32 to 0.53 when particle size has equal or <20 µm. These values were higher than those founded in this study with an $r = 0.10$. Another study [19], that worked with zooplankton fed with *Cylindrospermopsis* sp. *Anabaena* sp. and *Microcystis* sp. bacteria, founded when food concentration was under $5 \times 10^9 \mu\text{m}^3\text{L}^{-1}$, feed rates in zooplankton were low, but bacteria consume has zero values with *Anabaena* sp. and *Microcystis* sp. cyanobacteria.

Studies with *B. angularis* and *B. quadridentatus brevispinus* [20] from Tabasco, México, founded densities of 1000 org.500 mL⁻¹ when it was cultured at 10 g.L⁻¹ of salinity and fed with *Nannochloropsis oculata*. These densities were higher to our findings and also when compared without salinity cultures, which obtained 5,000 org.500mL⁻¹. Cultures with *B. angularis* fed with *Chlorella vulgaris* at 2.9 µg.mL⁻¹ concentration obtained densities of 51 ± 5 org.mL⁻¹, but when food concentration increase to 11.6 µg.mL⁻¹, it was obtained densities of 153 ± 15 org.mL⁻¹, with r values of 0.54 to 0.60 [21], these values were higher than those founded in this study. The growth rate (r) is an ecological tool used to quantify rotifer response to food type and population density in culture medium. When rotifer cultures are maintained in food concentrations below 1 µg.mL⁻¹, it only make that population have reproduction rate of zero or dye. The food that was applied to rotifer culture must contain enough energy to cover maintenance, growth and reproduction of organisms to avoid affect r values. The food quality also control abundance of rotifer population, but biochemical composition (amino acids, essential fatty acids and vitamins), also affect growth and reproduction not only for rotifer populations, but also zooplankton in general [21]. The r values must be affected also for ingestion rate and assimilation efficiency that rotifer populations have in culture medium. For this reason, r values obtained in this study cannot be taken as general parameter for this rotifer specie, but only as response to experimental conditions.

When chicken manure extract was used as food (bacteria) [22], combined with *Chlorella vulgaris* at density of 2.5×10^6 cel.mL⁻¹, to cultured *B. angularis* at 25 °C in darkness, authors founded that when it was added 2.0 mL.L⁻¹ of this extract, they obtained densities of 248.7 ± 16.4 org.mL⁻¹ and an r value of 0.71. It was observed that Estradiol hormone founded in chicken manure affecting growth rate of rotifers. Also, carbohydrates source from chicken manure was used as substratum for probiotic bacteria production (*Bacillus* sp.) which was used as food for rotifers and then increase their survival and reproduction. In our study, the only apply of bacteria as food source and not microalgae, produced that cultures didn't presented exponential growth and that's why bacteria were enough for maintenance of organisms. The chicken manure extract, rich in bacteria, can induce *B.*

angularis populations to parthenogenetic reproduction, which is primordial for these massive culture mediums and their use in larviculture of fishes and crustaceans [22].

5. Conclusion

Heterotrophic bacteria produced in Biofloc system, used as unique diet food for *B. angularis*, rotifer cannot reach high densities to obtain massive culture productions, but it can be use like complementary diet or maintenance food in absence of microalgae diet. It is important to consider that heterotrophic bacteria can be used as enriched diet because they can have probiotic characteristics useful to larviculture.

6. References

- Ogata Y, Tokue Y, Yishikawa T, Hagiwara A, Kurokura H. A Laotian Strain of the rotifer *Brachionus angularis* hold promise as a food source for small-mouthed larvae of freshwater fishin. *Aquaculture*. 2011; 312: 72-76.
- Garza-Mouriño G, Benítez-Díaz Mirón MI, Castellanos-Páez ME. Estudio morfométrico de *Brachionus angularis* en poblaciones de ambiente natural y en cultivo. *Revista Digital E-Bios*. 2011; 1:44-48.
- CRIP (Centro Regional de Investigación Pesquera). Protocolo de rotífero. Agencia de Desarrollos Tecnológicos. Manzanillo, Colima. 2014.
- Lavens P, Sorgeloos P. Manual on the production and use of live food for aquaculture. FAO Fisheries Technical Paper, 1996; 361: 295.
- Román-Reyes JC, Castañeda-Rodríguez DO, Castillo-Ureta H, Bojórquez-Domínguez R, Rodríguez-Montes de Oca GA. Dinámica poblacional del rotífero *Brachionus ibericus* aislado de estanques para camarón, alimentado con diferentes dietas. *Latin American Journal of Aquatic Research*. 2014; 42:1159-1168.
- Gutierrez-Wing M, Malone R. Biological filters in aquaculture: Trends and research directions for freshwater and marine applications. *Aquacultural Engineering*. 2006; 34:163-171.
- Castro-Nieto LM, Castro-Barrera T, De Lara-Andrade R, Castro-Mejía J, Castro-Mejía G. Sistemas biofloc: un avance tecnológico en acuicultura. *REVISTA DIGITAL E-BIOS*. 2012; 1:1-6.
- Cisneros R. Rendimiento poblacional del rotífero nativo *Brachionus* sp. cayman, utilizando diferentes enriquecedores. *Redalib*. 2011; 10:99-105.
- Lubzens E, Zmora O, Barr Y. Biotechnology and aquaculture of rotifers. *Hydrobiologia*. 2001; 446:337-353.
- Pascual Ey Yufera M. Crecimiento en cultivo de una cepa de *Brachionus plicatilis* O.F. Muller en función de la temperatura y la salinidad. *Investigaciones Pesqueras*. 1983; 47:151-159.
- Dhert Rotifers P, In Lavens P, Sorgeloos P. (Eds.), Manual on the production and use of live food for aquaculture. Technical paper FAO – ARC. 1996.
- Ma Q, Xi YL, Zhang JY, Wen XL, Xiang XL. Differences in Life Table demography among eight geographic populations of *Brachionus calyciflorus* (Rotifera) from China. *Limnologica*. 2010; 40:16-22.
- Hu H, Xi Y. Demographic parametrs and mixis of three *Brachionus angularis* Goss (Rotatoria) strains fed on different algae. *Limnologica*. 2008; 38:56-62.
- Wen XL, Xi YL, Yang YF, Zhang XA, Zhang G. Temperature is the key factor controlling population dynamics of *Brachionus angularis* in Lake Jinhu during

- Summer and Autumn, Journal of Freshwater Ecology. 2011; 26(2):277-286.
15. Hu HY, Xi YL. Differences in population growth and morphometric characteristics of three strains of *Brachionus angularis*. Journal of Freshwater Ecology. 2011; 21(1):101-108.
 16. Kostopoulou V, Vadstein O. Growth performance of the rotifers *Brachionus plicatilis*, *B. 'Nevada'* and *B. 'Cayman'* under different food concentrations, Aquaculture. 2007; 273:449-458.
 17. Mostary S, Shahidur RM, Amzad HM. Culture of rotifer *Brachionus angularis* Haver feeding with dried *Chlorella*. University Journal of Zoology, Rajshahi University. 2007; 26:73-76.
 18. Xi YL, Liu GY, Jin HJ. Population growth, body size, and egg size of two different strains of *Brachionus calyciflorus* Pallas (Rotifera) fed different algae. Journal of Freshwater Ecology. 2011; 17(2):185-190.
 19. Ka S, Mendoza-Vera JM, Bouvy M, Champalbert G, NGom-Ka R, Pagano M. Can tropical freshwater zooplankton graze efficiently on cyanobacteria? Hydrobiologia. 2012; 679:119-138.
 20. Rimber IJ, Páramo OS, Arias RL, Marquez CG, Segers H, Alvarez GCA *et al.* Freshwater rotifer (part II) a laboratory study of native freshwater rotifers *B. angularis* and *B. quadridentatus brevispinus* from Tabasco. Kukulkab' Revista de Divulgación. 2012; 18(35):31-38.
 21. Espinoza-Rodríguez CA, Rivera-De la Parra L, Sarma SSS, Nandini S. Effect of food density of *Pseudokirchneriella subcapita* and *Chlorella vulgaris* on the population growth rates of four brachionid rotifers. Inland waters. 2014; 4:271-278.
 22. Ochieng OE, Hagiwara A. Effect of chicken manure extract on the population growth, mixis induction and body size of the freshwater rotifer *Brachionus angularis* Gosse 1851. Asian Fisheries Science. 2015; 28:174-185.