UNED Research Journal / Cuadernos de Investigación UNED
ISSN: 1659-4266
cuadernosuned@gmail.com
Universidad Estatal a Distancia
Costa Rica

Ortega-Salas, A. A.; Reyes, C. I.; Reyes-Bustamante, H.
Population growth and cyst production of the rotifer Brachionus plicatilis (Monogonta: Brachionidae) fed with three different diets
UNED Research Journal / Cuadernos de Investigación UNED, vol. 5, núm. 2, enero-junio, 2014, pp. 195-199
Universidad Estatal a Distancia
San José, Costa Rica

Population growth and cyst production of the rotifer *Brachionus plicatilis* (Monogonta: Brachionidae) fed with three different diets

A. A. Ortega-Salas, C. I. Reyes and H. Reyes-Bustamante

Unidad Académica Mazatlán, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Calzada Joel Montes Camarena s/n Mazatlán, 82040, Sinaloa, México; ortsal@ola.icmyl.unam.mx

Received 12-II-2013 Corrected 29-IV-2013 Accepted 6-VII-2013

ABSTRACT

This study compares the effect of different diets on population growth, fecundity rate, production, and cyst hatching efficiency of the rotifer *Brachionus plicatilis*. Microalgae were cultured using filtered and sterilized seawater at 25 and 26°C. The volume of the containers for algae, bread yeast, and rotifer was duplicated daily from 31mL to 16L in nine days. Rotifers were cultured at 25°C and 35‰ salinity. The mean values of daily population growth rate for the rotifers fed on *C. muelleri*, *I. galbana* and bread yeast were 0,91, 0,89 and 0,87 daily counts, respectively. After 10 cultured days, fecundity was 72%, 63% and 36% using *C. muelleri*, *I. galbana* and bread yeast, respectively. The highest mean value for cysts production was 4 064 000 cysts found from 63 243 (1,55%) rotifers fed on *C. muelleri*. Hatching efficiency after 36 hours of cysts from rotifers fed *C. muelleri* was 51 500 (94,3%) new borns, followed by 45 000 (93,3%) with *I. galbana*, and 31 000 (92,6%) with bread yeast. *C. muelleri* is a good food source for *B. plicatilis*.

KEY WORDS

Fecundity, feeding, hatching, salinity, temperature

RESUMEN

Este estudio compara el efecto de la dieta alimenticia en el crecimiento poblacional, la tasa de fecundidad, producción y eficiencia de desove de quistes en el rotífero *Brachionus plicatilis*. Para la producción de microalgas, el agua marina se filtró sucesivamente y se mantuvo entre 25° y 26°C. El volumen de los contenedores para algas, levadura y rotíferos se duplicó diariamente de 31mL a 16L en nueve días. Los rotíferos se mantuvieron a temperaturas de 25°C y salinidad de 35‰. La tasa de crecimiento de los rotíferos por día fue de 0,91 alimentados con *C. muelleri*, 0,89 con *I. galbana* y de 0,87 con levadura. La fecundidad de los rotíferos 10 días fue de 0,72 cuando fueron alimentados con *C. muelleri*, 0,63 con *I. galbana* y 0,36 con levadura. La producción más alta de quistes fue de 63 243 (1,55%) a partir de 4 064 000 rotíferos cuando se alimentan con *C. muelleri*. La eficiencia de desove de quistes de rotíferos alimentados con *C. muelleri* fue de 51 500 (94,6%) de neonatos después de 36 horas, 45 000 (93,3%) con la dieta de *I. galbana* y de 31 000 (92,6%) con levadura. *C. muelleri* mostró ser una buena fuente de alimentación.

PALABRAS CLAVE

Alimentación, desove, fecundidad, temperatura, salinidad

In order to expand the practice of marine finfish larvae rearing, it is necessary to increase and stabilize the production of live feed. The technology is based on the use of highly concentrated algal biomass as food for the rotifers. Villegas, Millamena and Escritor (2008) studied the effects of three selected algal species, *Tetraselmis tetrahele*, *Isochrysis galbana*, and marine *Chlorella* sp., on the population growth of *Brachionus plicatilis* after three, five and seven days of culture. The rotifers fed on *T. tetrahele* showed growth with a mean peak density of 92,5 individuals per mL. This result was superior to those fed on *I. galbana* (48,2 individuals per mL) and *Chlorella* sp. (47,2 individuals per mL) in five days. Rotifers are mass-cultured at 10 000-30 000 individuals mL-1 by feeding them exclusively on condensed freshwater *Chlorella*. The cultures are generally supplied with O_2 at a constant rate; and the pH is adjusted to 7 by addition of HCI to avoid an increase of free ammonia in the culture system, substituted rotifers for *Artemia* in larval fish culture. He is using a new, continuous culture system that appears to be reliable and stable. It is still in the development phase, but he is averaging about 8Kg of wet weight rotifers per day. Rotifer production has very significant economies of scale. Labor cost is almost independent of production levels. He is

using yeast and other nutrients in a continuous production system with L-type rotifers. Apparently large rotifer production systems will solve the cost of producing live Artemia nauplii; hence, it is important to know the biology of rotifers and their nutritional requirements to optimize their culture. Since the supply of rotifers does not always meet the demand, it is necessary to apply techniques to produce high quality cysts that can be used to initiate rotifer culture systems on a large scale.

The aim of this study was to compare the population growth rate of *B. plicatilis*, production of cysts, fecundity rate (Release eggs/Eggs in each female in the sample), and efficiency of hatching when the rotifers where fed on the microalgae *Chaetoceros muelleri* Lemmermann, or *Isochrysis galbana* Bruce, Knight and Parke, or baker´s yeast *Saccharomyses cerevisiae* strain Lalvin EC1118 (Prise de mousse) as food.

MATERIAL AND METHODS

This study was performed at the Aquaculture Laboratory, Facultad de Ciencias del Mar, Universidad Autónoma de Sinaloa, using two rooms: one, to culture microalgae, which was provided with 13 white-light lamps from 40 to 75W, and the other, for the culture of rotifers. Each room has a water aeration system for culturing *C. muelleri* (CHGRA) (Lora-Vilchis, Cordero & Voltolina, 2004), *I. galbana* microalgae and rotifers.

There are two main sizes for *B. plicatilis*, small (S) and large (L); they differ in the lorica length, which is 100-210µm (mean 160µm) for S type *B. rotundiformis* (Gómez, 2005) and 130-340µm (mean 239µm) for L type. In this study the L type was used. The rotifer strains were isolated from an oasis in San Pedro, Baja California (Ramírez-Sevilla, Rueda Jasso, Ortiz-Galindo & González-Acosta, 1991). Their cysts are yellow-brown, rounded, and wrinkled on the surface, with diameters of 10µm (25%), 11µm (60%) and 12µm (15%).

The seawater for culturing microalgae was filtered successively through filters of 5,0, 1,0, and 0,45µm (Reyes, 2003), ultraviolet radiation was used to sterilize the water; controlled temperature between 25° and 26°C was maintained to permit the rotifer population grow adequately. The rotifers were initially maintained in 500mL flasks at a temperature of 25°C and salinity of 35‰ and fed on microalgae or bread yeast. *C. muelleri* measures between 4 and 7µm in diameter by 6 to 8µm in length, whereas *I. galbana* measures 8µm in diameter by 11 to 13µm in length (Ortega, 1984; Trujillo & Voltolina 1994). The production of microalgae according to Matthiessen and Toner (1966) method, doubling the volume of the containers daily from 31mL to 16L in nine days.

The rotifer culture started 10 to 20organisms/mL. The growth of the rotifer population was assessed by daily counts according to Yúfera and Pascual (1980) with the formula Ke = (ln Nt - ln N0)/ T, where N0 is the number of organisms at the start of a period of T days, and Nt the number after T days. The population growth was assessed in triplicate each sampling. The rotifers were fed each one of the two selected microalgae at a density of $1x10^6$cells/mL, and bread yeast dissolving 1g in 20L seawater each day. The volume of the rotifers culture was doubled daily from 31mL tubes to 16L containers in nine days. Each day the rotifers were concentrated in a sieve and transfer to the next volume containing the algae's and the bread yeast.

The fecundity rate $A_f = H/n$ was estimated from the number of eggs released per eggs in each female, where H is the total number of eggs released and n is the number of females which have eggs in the sample (Table 1).

Production of cysts from three essays: rotifers were filtered into 18L flask $2x10^6$cels/ml density of the designed microalgae for rotifers to feed, daily, also rotifers were filtered in 1g of bread yeast in 18L flask, until density reached more than 100rot/mL, then feeding stopped; sexual reproduction started (mictic), 5 days later rotifers were filtered using a 60µm mesh, one 20^{th} part of the volume was evaporated by light sun rays then cysts were cleaning using brine water and mixed with the

TABLE 1
Final concentration of *B. plicatilis* with different foods*

	E. in Female/mL	L. Eggs/mL	Rotif+Eggs	Fecundity
		C. muelleri		
Mean	79,6	30,7	108	0,34
SD	44,5	23,8	65,8	0,15
		I. galbana		
Mean	70,2	24	94,2	0,29
SD	32,4	26,7	56	0,2
		Bread yeast		
Mean	58,7	18,1	76,8	0,32
SD	27,5	8,7	35,7	0,08

*Considering increase of volume, eggs in female, number of release eggs, rotifers plus the eggs and fecundity rate in an increasing volume in 10 days

substratum, then stir up for one minute, the cyst floated and were counted.

To evaluate the percentage of hatching, groups of 100 well preserved cysts were settled in a Petri dish with sterilized water, a 60W lamp was placed at 60cm for 24h, and the neonates were counted and separated until no more were born. The cysts were also decapsulated to obtain more neonates; they were settled with a mixture of 12mL of sodium hypochlorite with 0,6mL of sodium hydroxide at 40% for 15min, then watered, and 3mL of sodium thiosulfite was added to neutralize them, and then they were watered again (US Patent 7258890 - Process for decapsulating crustacean or rotifer eggs) (Table 2).

Efficiency and rate of hatching: 0,1g of cysts was settled in a 1L flask with light and aeration for 24h at 25°C; using a 1mL micropipette, neonates were count and extrapolate to 1g cysts. The rate of hatching after 12h, the first count was made each hour in three samples until 90% of cyst hatched. T0 (10%) = time when the first neonate hatched, until T_{90} (90 %) $T_s = T_{90}-T_0$ (Table 2) where Ts is the synchronized time (Table 2).

The ANOVA and Kruskal Wallis statistical methods were used.

RESULTS

Mean daily population growth rate ke for rotifers was 0,91(±0,09SD) fed on *C. muelleri*, 0,89(±0.05SD) on *I. galbana* and 0,87(±0,09SD) on bread yeast; these rates showed no significant differences between *C. muelleri* and bread yeast, or between *C. muelleri* and *I. galbana* (P=2,77) under a crescent culture (Fig. 1 and 2). The average fecundity rate was relatively higher with *C. muelleri* 0,35(±0,15SD) as compared to bread yeast 0,29(±0.2SD) and *I. galbana* 0,33(±0,08SD), but no significant differences were fed among diets (Table 1).

Table 2 shows the mean number of rotifers plus eggs in the 16 containers on day ten was 4 064 000 ±741 717 fed with C muelleri, 3 221 333±720 059 with I. galbana 2 762 667±345 516 and with bread yeast (Fig. 1 and 2).

The highest mean production of cysts was 63 243(1,55%); *C. muelleri* was used as food. The percentage of hatching was higher at 24h time intervals. Efficiency of hatch extrapolate to 1g of cysts from rotifers fed with *C. muelleri* was 51 500(94,3%) neonates at 36h (Table 2). ANOVA showed no significant differences between *C. muelleri* and bread yeast, or between *C. muelleri* and *I. galbana* (F2,27, P<0,05). The percentage of hatching varied

TABLE 2

Production of cysts, interval of hatching, hatching efficiency, and percentage of hatching from cysts after *C. muelleri, I. galbana,* or bread yeast had been fed to rotifers**.**

	Number of rotifers	Cysts produced	% cysts	% hatched after time intervals			Hatching efficiency/g	% of hatching
				12h	24h	36h		
					C. muelleri			
Mean	4 064 000	63 243	1,55	17,3	75	7,6	51 500	94,3
SD	741 717	3 568	0,35	3,51	5,2	2,5	2 500	2,52
					I. galbana			
Mean	3 221 333	15 402	0,47	18	75	7	45 000	93,3
SD	720 059	2 329	0,21	2,65	2	1	2 000	3
					Bread yeast			
Mean	2 762 667	33 383	1,21	16	73	10,6	31 000	92,6
SD	345 516	2 770	0,12	3	4,04	3,21	6 557	2,52

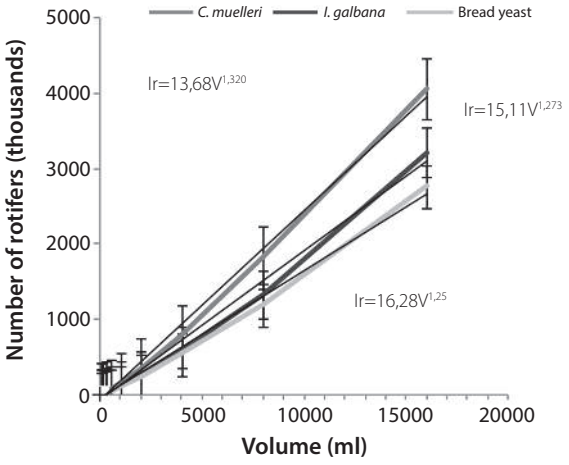

FIG. 1. Number of rotifers (Nr) as function of the increasing volume of the containers fed on microalgae and bread yeast. Regression lines and standard errors are shown.

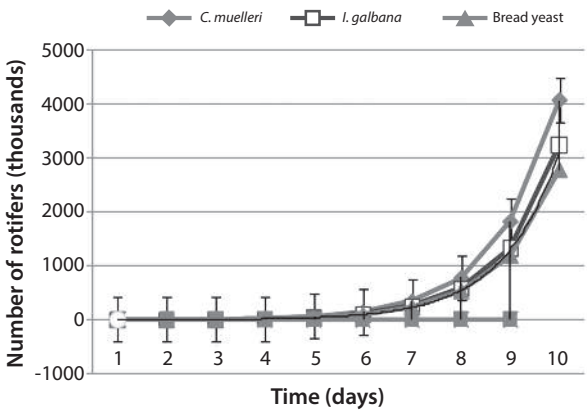

FIG. 2. Number of rotifers (Nr) as function of the increasing time fed on microalgae or bread yeast. Equations of the regression lines and standard error are shown.

from 92,6% fed rotifers bread yeast to 94,3% fed *C. muelleri*. This percentage decreases from about 90 in 15 days to less than 60 in 60 days.

Fecundity and cyst production showed that *C. muelleri* to be better food.

DISCUSSION

The size of the buccal cavity of a rotifer depends on the overall body size. The relationship between maximum size of the food ingested (Tm) and lorica length (L) is as follows: Tm= 0,090L–0,033 (Hino & Hirano, 1980). For rotifers between 250 and 300 µm, the maximum food ingested measured between 22 and 27µm. In the present study, *B. plicatilis* had good results fed on *C. muelleri*, which in this case measured less than 10 µm (Trujillo & Voltolina, 1994).

The rate of ingestion increases with the concentration of the food up to a maximum level, then the ingestion rate remains constant (Hirata & Mocawa, 1983). In the present study the hatching rate was 94,3% when food was *C. muelleri*.

Minkoff, Lubzens and Kahan (1983) mentioned that at 9‰ salinity, hatching of *B. plicatilis* was optimal (40–70%) when the temperature was 10–30° C and decreased linearly with rise in incubation temperature. Light was obligatory for termination of dormancy. At 15°C resting eggs incubated over a salinity range of 9–40‰ showed optimal hatching at 16o/oo. In the present study at 25ºC and salinity of 35‰ cysts were given light and aeration for 24h.

Frequent densities of rotifers in commercial cultures are 2 500rotifers/mL in recirculation system (Suantika, Dhert & Sorgeloos, 2003), although ultra densities of more than 1,6x105/mL have been reported (Yoshimura, Tanaka & Yoshimatsu, 2003). In the present study, the highest densities reached were of 254rotifers+ eggs/mL fed on *C. muelleri*, and the lowest was 172rotifers+eggs/mL fed with bread yeast after 9 days of culture in 16L. *C. muelleri* showed to be better food.

ACKNOWLEDGEMENTS

We thank A. Nuñez P. and S. Rendón R. for technical support.

REFERENCES

Gómez, A. (2005). Molecular ecology of rotifers: from population differentiation to speciation. *Hydrobiology*, 546, 83–99. doi: 10.1007/s10750-005-4104-7

Hino, A., & Hirano, V. (1980). Relationship between body size of the rotifer *Brachionus plicatilis* and the maximum size of particles ingested. *Bulletin of the Japanese Society of Scientific Fisheries*, 46(10), 1217–1222.

Hirata, H. S., & Mocawa, T. (1983). Continuous culture of the rotifer Brachionus plicatilis fed recycled algal diets. *Hydrobiology*, 104, 71-75.

Lora-Vilchis, M. C., Cordero, B. E., & Voltolina, D. (2004). Growth of *Artemia franciscana* fed *Isochrysis* sp. and *Chaetoceros muelleri* during its early life stages. *Aquaculture Research*, 35, 1086-1091. doi: 10.1111/j.1365-2109.2004.01123.x

Matthiessen, C. G., & Toner, R. G. (1966). *Possible shellfish industry of Martha´s Vineyard, Duke´s County Massachussetts.* Edgartown, Massachussets, USA: Marine Research Foundation.

Minkoff, G., Lubzens, E., & Kahan, D. (1983). Environmental factors affecting hatching of rotifer (*Brachionus plicatilis*) resting eggs. *Hydrobiology*, 104(1), 61-69.

Ortega, M. M. (1984). *Catálogo de algas continentales recientes de México UNAM*. México: UNAM.

Ramírez-Sevilla, R., Rueda Jasso, R., Ortiz-Galindo, J. L., & González-Acosta, B. (1991). Metodología para el cultivo del rotífero *Brachionus plicatilis. Investigaciones Marinas CICIMAR*, 6(2), 287-290.

Reyes C. (2003). *Cultivo experimental del rotífero Brachionus plicatilis con tres diferentes dietas y el análisis de la calidad de los quistes.* Tesis de licenciatura. Facultad Ciencias Marinas, Universidad Autónoma de Sinaloa, México.

Suantika, G., Dhert N. P., & Sorgeloos, M. P. (2003). High-density production of the rotifer Brachionus plicatilis in a recirculation system: consideration of water quality, zootechnical and nutritional aspects. *Aquaculture Engeniering*, 21, 201-213. doi: 10.1016/S0144-8609(99)00031-X

Trujillo, M., & Voltolina, D. (1994). *Cultivos de microalgas para la acuicultura.* Departamento de acuicultura. Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California, México.

US Patent 7258890 - *Process for decapsulating crustacean or rotifer eggs*. US Patent Issued on August 21, (2007). Estimated Patent Expiration Date: May 13, 2023.USA.

Villegas C. T., Millamena, O., & Escritor, F. (2008). Food value of *Brachionus plicatilis* fed three selected algal species as live food for milkfish, *Chanos chanos* Forsskal, fry production. *Aquaculture Research*, 21(2), 213 – 220.

Yoshimura, K., Tanaka, K., & Yoshimatsu, T. (2003). Anovel culture system for the ultra-high-density production of the rotifer, *Brachionus rotundiformis* a preliminary report. *Aquaculture*, 227, 165-172.

Yúfera, M., & Pascual, E. (1980). Estudio del rendimiento de cultivo del rotífero *Brachionus plicatilis. Hydrobiology Society URRS*, 44(2), 361-368.