Influence of Food (*Chlorella vulgaris*) Concentration and Temperature on the Population Dynamics of *Brachionus calyciflorus* Pallas (Rotifera) Isolated from a Subtropical Reservoir in Mexico

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Abstract. *In this study we have analyzed the population growth of the rotifer* Brachionus calyciflorus *subjected to different conditions of temperature (25° C and 30° C) and algal concentration (namely, 1.0 X 10^6, 2.0 X 10^6 and 4.0 X 10^6 cells ml^{-1}). We found that peak population densities were reached at around day 6 at 30° C but between day 9 to 13 at 25° C. The lowest r value recorded in the study was 0.30±0.03 at a food concentration of 1 X 10^6 cells ml^{-1} at 25° C and the highest population growth rate (0.47±0.01) at a food concentration of 4 X 10^6 cells ml^{-1} at 25° C. Both temperature and food concentration had a significant impact on the maximum population density reached, as well as the time necessary to reach the average peak abundance or the rate of growth per day (r).*

Introduction

The rotifer *Brachionus calyciflorus* has been extensively used as an indicator of pollution (Joaquim-Justo *et al.*, 1995), as a bioassay organism (Snell and Janssen, 1995), and as food for rearing larval fish (Awaiss *et al.*, 1992). This species has also been recently included as a standard bioassay organism by the American Society of Testing and Materials in the USA (ASTM, 1991). *B. calyciflorus* is a widely distributed rotifer found in many freshwater bodies around the world including Mexico (Koste, 1978). Since its body size and population growth rates are normally controlled by the trophic conditions and temperatures of the ambient waters (Halbach, 1970; Bennett and Borass, 1989), different authors have reported different growth rates for the same species (Bennett *et al.*, 1993; Rothhaupt, 1993).

Different strains of *B. calyciflorus* show differences in their responses to food availability and temperature. In addition, workers elsewhere have used different food types and densities (Gilbert, 1970; Starkweather and Keller, 1983; Weithoff and Walz, 1995; Sarma *et al.*, 1997). Even if we express all food types in terms of cell number, dry weight, carbon content or caloric value, the results are likely to vary more than expected because a rotifer's ability to digest a particular algal type also depends on the physical structure and chemical constitution of the algae (Pourriot, 1965). Thus, from the literature, one finds the population growth rate of *B. calyciflorus* under optimal conditions ranging from 0.1 to 2.0 per day (Sarma, 1991). It is therefore not known how a given rotifer species (or strain) will increase with increasing food concentration under varying temperatures. This information is necessary for successful management of rotifer cultures so that they can be effectively used in aquaculture for rearing fish larvae.

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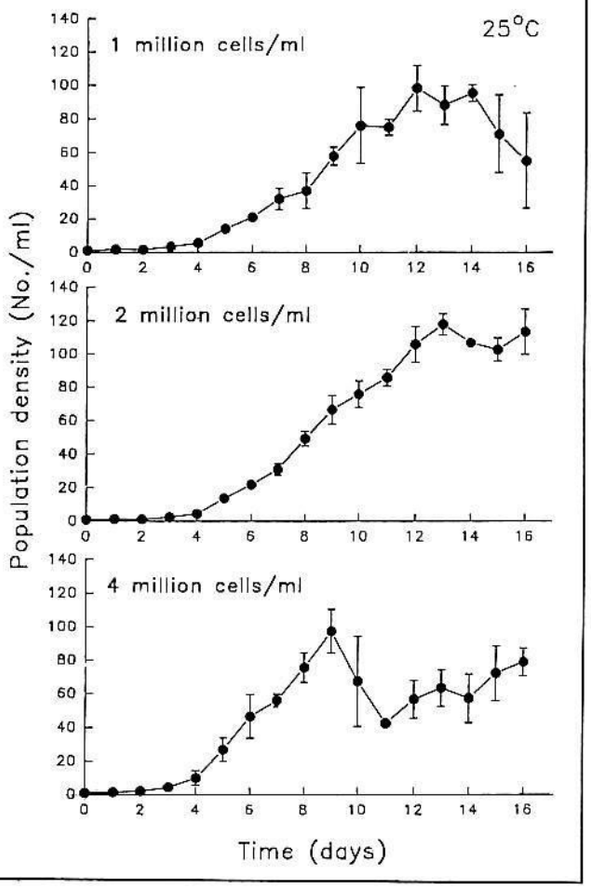
I. Material and Methods

The rotifer *Brachionus calyciflorus* (average adult length, excluding spines 185 ± 12 μm) was originally isolated from Lake Chapultepec (Lago Viejo) (Mexico City) and successfully cultured in the laboratory using the single-celled green algae (*Chlorella vulgaris*, average cell diameter: 5.48 ± 1.21 μm) as the exclusive food (Sarma *et al.*, 1997). *Chlorella* was mass cultured non-axenically in transparent bottles (Sarma, 1996) using Bold Basal medium (Borowitzka and Borowitzka 1988).

Rotifers were routinely fed algae at a density of approximately 2 X 10^6 cells ml^{-1} once a day. We used EPA (Anonymous, 1985) medium (using de-ionized water) for culturing rotifers. In our routine cultures we were able to obtain rotifers at a density of about 100 ind. ml^{-1}. However, we generally maintained the population below 50 ind. ml^{-1} in order to reduce the possibility of male production and consequent population decrease. For routine feeding as well as for experiments, we used log phase algae, centrifuged at 4000 rpm, rinsed in distilled water and resuspended in EPA medium. The density of algae was estimated daily using a haemocytometer.

FIGURE 1. POPULATION GROWTH OF *B. CALYCIFLORUS* AT 25° C AT THREE DIFFERENT ALGAL (*CHLORELLA VULGARIS*) FOOD CONCENTRATIONS IN RELATION TO TIME. VALUES SHOWN ARE MEAN ± SD BASED ON THREE REPLICATE RECORDINGS.



For experiments, we used 25 ml capacity transparent vials containing 20 ml of the EPA medium. All experiments were conducted in thermostatically controlled water-baths set at the desired temperatures. The initial pH of the medium was adjusted to 7.5. In all, we used 18 test vessels. For each food concentration-temperature combination, we used 3 replicates. Based on a preliminary test, we chose two test temperatures, namely 25 and 30° C. Three algal concentrations (namely, 1.0 X 10^6, 2.0 X 10^6 and 4.0 X 10^6 cells ml^{-1}) were used. Thus, the experimental design consisted of 18 test vessels (2 temperatures X 3 algal densities X 3 replicates = 18). Into each of the 18 test vessels, we introduced *B. calyciflorus* at a density of 1 ind. ml^{-1}. The initial population of rotifers, counted individually, consisted of a mixed age-group obtained from a mass-culture tank during the exponential phase of their growth. The test vessels were maintained in diffuse and continuous fluorescent illumination. No aeration was provided to the test vessels.

For counting rotifers, we used one of the two methods: a) whole count when the density of rotifers was less than 5 ind. ml^{-1} or b) aliquot subsamples of 1-5 ml volume when the density was greater than 5 ind. ml^{-1}. For each replicate, we counted at least 3 subsamples. Following inoculation, we estimated the population density every day until most replicates completed one population cycle. Thus, the experiment was terminated after day 16. Everyday, after estimating the population density, rotifers from all replicates were transferred to fresh EPA media containing algae at desired density and maintained at the chosen temperature.

For estimating the population density, we counted only live rotifers. Males did not appear during the test period. Population density of rotifers was expressed as number per ml. For estimating the population growth rate (r) of rotifers, we used the following formula (Poole, 1974):

$r = (\ln N_t - \ln N_0)/t$

where

N_0 = Initial population density

N_t = Population density after the time t

t = Time in days.

II. Results

The population growth of *B. calyciflorus* was significantly influenced by both food concentration and temperature (figures 1 and 2). The peak abundance of the population recorded in this study varied from 97 ± 4 - 118 ± 6 at 25° C and 36 ± 2 - 83 ± 11 at 30° C respectively (table 1). The population growth followed a logistic growth, reaching an asymptote at around day 6 at 30° C but was

delayed to between day 9 and 13 at 25° C. The rate of population increase per day (r) increased with increasing temperature and food concentration ($p < 0.01$, 2-way ANOVA). However, their interaction was not statistically significant ($p > 0.05$, 2-way ANOVA; table 2). The lowest r value recorded in the study was 0.30 ± 0.03 at a food concentration of 1 X 10^6 cells ml^{-1} at 25° C and the highest population growth rate (0.47 ± 0.01) at a food concentration of 4 X 10^6 cells ml^{-1} at 25° C (figure 3). At all the food concentrations tested, maximum population densities and average peak densities reached were higher at 25° than at 30° C. Temperature, food concentration and their interaction had a significant impact on the maximum population density reached and the time taken to reach the average peak abundance ($p < 0.01$, 2-way ANOVA, table 2).

III. Discussion

Our strain of *B. calyciflorus* was isolated from a lake located in high altitude where the temperature variation is closer to subtropical conditions (water temperature rarely exceeds 30° C) (Alcocer and Escobar, 1996). The two temperature ranges used here thus reflect annual average (which is closer to 25° C) and above average conditions. From the population growth curves, it is evident that rotifers grown under 25° C reached higher densities when compared to those under 30° C. However, the rate of population increase (r) showed that 30° C resulted in rapid population growth when compared to 25° C. Sarma *et al.* (1996) have grown *B. calyciflorus* (an African strain) in *Scenedesmus* at concentrations ranging from 0.5 X 10^6 cells ml^{-1} to 40.5 X 10^6 cells ml^{-1}. They found that the population growth rate per day varied from 0.792 ± 0.063 to 1.492 ± 0.129. In the present study, we obtained the r values which were on the lower side of this range. Sarma *et al.* (1997) have recently grown *B. calyciflorus* (also obtained from Lake Chapultepec) in four concentrations of *Chlorella vulgaris* ranging from 0.5 X 10^6 cells ml^{-1} to 4 X 10^6 cells ml^{-1} but at one temperature (27° C). The highest r value (0.82 ± 0.03) was obtained when the highest food density was offered. In the present investigation too, it is evident that the r value increased with increasing food density at any particular temperature. The fact that we got a lower r values than those reported in Sarma *et al.* (1997) at comparable food concentrations could be due to the differences in the initial inoculation density (ranging from 0.5 to 40.5 ind. ml^{-1}) of rotifers in their test vials.

There are several ways of expressing the maximum density reached by rotifers under culture conditions. Some of them are: a) mean plateau density which is an average of several points on the asymptotic phase of the population growth curve (Dumont *et al.*, 1995); b) average peak density, which is the highest value of popula-

FIGURE 2. POPULATION GROWTH OF *B. CALYCIFLORUS* AT 30° C AT THREE DIFFERENT ALGAL (*CHLORELLA VULGARIS*) FOOD CONCENTRATIONS IN RELATION TO TIME. VALUES SHOWN ARE MEAN ± SD BASED ON THREE REPLICATE RECORDINGS.

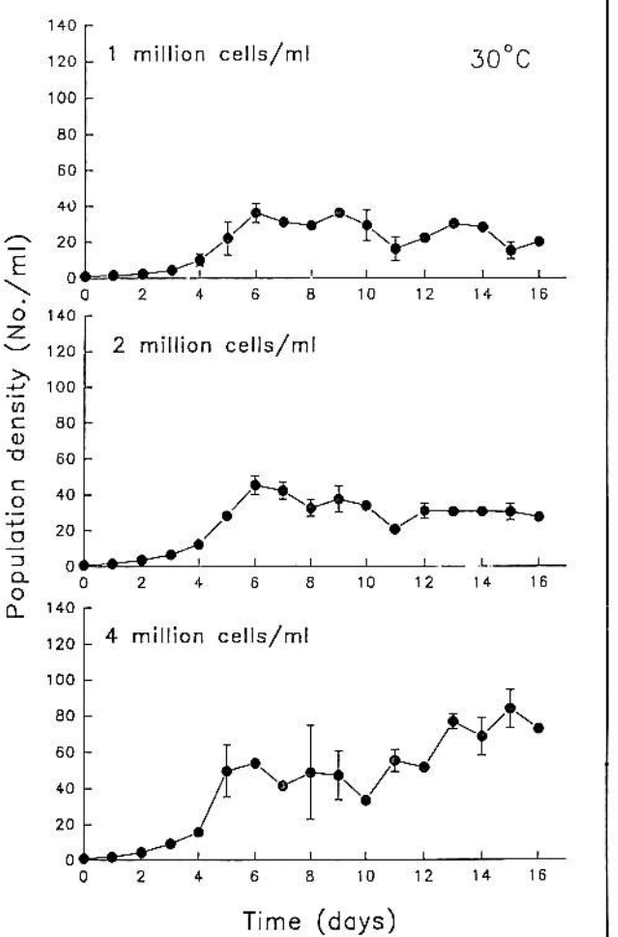


TABLE 1

INFORMATION ON THE MAXIMUM POPULATION DENSITY AND PEAK POPULATION DENSITY OF *BRACHIONUS CALYCIFLORUS* IN RELATION TO FOOD DENSITY AND TEMPERATURE. DATA WERE ALSO GIVEN ON THE DAYS AT WHICH MAXIMUM POPULATION DENSITY AND PEAK DENSITY WERE OBTAINED. FOR EXPLANATION, SEE TEXT.

TEMP. (° C)	FOOD DENSITY (X 10^6 CELLS ml^{-1})	MAX. POPULATION DENSITY (NO./ML) Y ± S.D.	DAY AT MAX. POPULATION DENSITY Y ± S.D.	AVERAGE PEAK DENSITY (NO./ML) Y ± S.D.	DAY AT AVERAGE FIRST PEAK DENSITY
25° C	1	104 ± 5	12.7 ± 1.2	98 ± 14	12
	2	118 ± 6	13.0 ± 0.0	118 ± 6	13
	4	97 ± 13	9.0 ± 0.0	97 ± 4	9
30° C	1	38 ± 4	8.0 ± 1.7	36 ± 2	9
	2	45 ± 5	6.0 ± 0.0	45 ± 5	6
	4	86 ± 8	14.7 ± 0.6	83 ± 11	8

TABLE 2

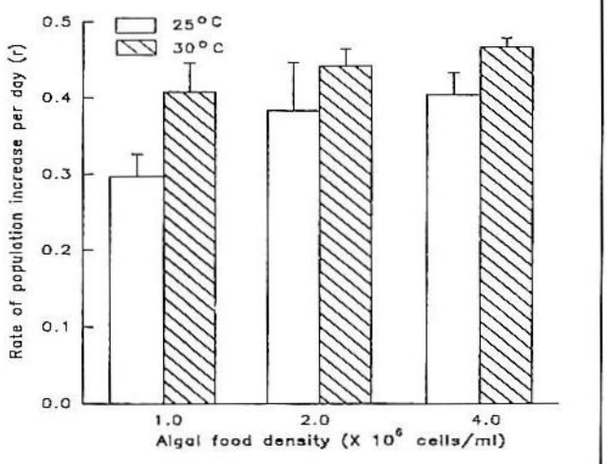
Two-way Statistical Analysis (ANOVA) of Selected Population Variables in *B. calyciflorus*.

Population Variable	Parameter	DF	SS	MS	F Ratio	P
Maximum Population Density						
	Food Concentration (F)	2	1279.67	639.84	7.39	0.01
	Temperature (T)	1	10115.13	10115.13	116.77	0.001
	Interaction (F x T)	2	2822.02	1411.01	16.29	0.001
	Error	12	1039.45	86.62		
Day at Maximum Density						
	Food Concentration (F)	2	19.00	9.50	11.40	0.01
	Temperature (T)	1	16.06	16.06	19.27	0.01
	Interaction (F x T)	2	131.44	65.72	78.87	0.001
	Error	12	10.00	0.83		
Population Growth Rate (*r*)						
	Food Concentration (F)	2	0.022	0.011	8.46	0.01
	Temperature (T)	1	0.027	0.027	20.77	0.001
	Interaction (F x T)	2	0.003	0.0015	1.15	0.350
	Error	12	0.016	0.0013		

tion abundance on any one particular day when all replicates are combined (Dumont and Sarma, 1995); this is normally used by most workers and is evident from the population growth curves; c) maximum population density which is a third type of expressing the highest population density using several replicates. In this case, since all replicates of a particular test condition do not show the highest value on the same day, individual replicates with highest values (regardless of day) are pooled and expressed as mean. This approach was followed by Iyer and Rao (1996) on the population growth of the predatory rotifer *Asplanchna intermedia*. Sarma *et al.* (1996) have also mentioned this in their work on the competition between two herbivorous rotifers (*B. calyciflorus* and *Anuraeopsis fissa*).

Table 1 gives information on the maximum population density and average peak density in relation to time. It is obvious that these two values are closely related. The values would become exactly the same when all replicates show highest value of the population abundance on one particular day and in a single peak, which is generally rare (e. g., Sarma and Rao, 1990).

FIGURE 3. The Rate of Population Increase per day (*r*) of *B. calyciflorus* Subjected to Three Algal (*Chlorella vulgaris*) Food Levels (1, 2 and 4 x 10^6 Cells ml^{-1}) at 25° C (Blank) and 30° C (Back Slash). Values Shown are Mean ± SD Based on Three Replicates.



The peak density values recorded here were comparable to those found in other studies on this species. Sarma *et al.* (1996) have shown that *B. calyciflorus* reached the peak abundance of 860 ± 69 ind. ml^{-1} at 40.5 X 10^6 cells ml^{-1}, but at comparable food levels of 1, 2 and 4 X 10^6 cells ml^{-1}, based on their regression equation (intercept and slope wrongly interchanged), the values appear to be lower (4, 19, and 48 ind. ml^{-1}, respectively, under the above food levels) compared to the present work (figures 1 and 2). On the other hand, Sarma *et al.* (1997) have reported the peak density of 137 ± 15 ind. ml^{-1} at 27° C when the food density was 4 X 10^6 cells ml^{-1}. This value is close to the present study (118 ± 6 ind. ml^{-1}) under the same food level of the same algal species at 25° C. Thus, it appears that the strain level differences could reflect on the population variables.

An increase in the ambient temperature from 25° to 30° C reduced both the maximum population density and the average density. This may be explained on the basis of a) strain adaptations (in the present case, it is a low-temperature adapted strain and hence higher temperatures did not enhance the population growth optimally; the annual average temperature from the Lago Viejo is about, 17 ± 2° C (see Alcocer, 1988); and b) on basis of metabolic demands which are higher at higher temperature (Sarma and Rao, 1990). Thus, the present study indicated that this strain of *B. calyciflorus* was better adapted to the lower temperature provided (25° rather than 30° C) regardless of the food level.

BIBLIOGRAFÍA

Alcocer, J. (1988). *Caracterización hidrobiológica de los lagos de Chapultepec, México.* Tesis de maestría en Ciencias del Mar. UNAM, México.

Alcocer, J. and Escobar, E. (1996). "Limnological Regionalization of Mexico", in *Lakes & Reservoir: Res. Manag.* Vol. 2, pp. 55-69.

Anonymous (1985). *Methods of Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms.* US Environment Protection Agency. EPA/600/4-85/013.

ASTM (1991). "Standard Guide for Acute Toxicity Tests with the Rotifer *Brachionus*", in *Annual Book of ASTM Standards.* Vol. 11. 04, E1440, American Society for Testing and Materials, Philadelphia, PA, USA.

Awaiss, A., Kestemont, P. and Micha, J. C. (1992). "Nutritional Suitability of the Rotifer, *Brachionus calyciflorus* Pallas for Rearing Freshwater fish Larvae", in *J. Appl. Ichthyol.* Vol. 8, pp. 263-270.

Bennett, W. N. and Borass, M. E. (1989). "A Demographic Profile of the Fastest Growing Metazoan: a Strain of *Brachionus calyciflorus* (Rotifera)", in *Oikos.* Vol. 55. pp. 365-369.

Bennett, W. N., Boraas, M. E. and Seale, D. B. (1993). "Turbidostat Culture of *Brachionus calyciflorus*: an Experimental System to Assess Biological Limits on Population Growth", in Walz, N (ed). *Plankton Regulation Dynamics. Experiments and Models in Rotifer Continuous Cultures.* Ecological Studies. Vol. 98. pp. 77-86. Springer Verlag, Berlin.

Borowitzka, M. A. and Borowitzka, L. J. (1988). *Micro-algal Biotechnology.* Cambridge University Press, London.

Dumont, H. J. and Sarma, S. S. S. (1995). "Demography and Population Growth of *Asplanchna girodi* (Rotifera) as a Function of prey (*Anuraeopsis fissa*) Density", in *Hydrobiologia.* Vol. 306, pp. 97-107.

Dumont, H. J., Sarma, S. S. S. and Ali, A. J. (1995). "Laboratory Studies on the Population Dynamics of *Anuraeopsis fissa* (Rotifera) in Relation to food Density", in *Freshwater Biol.* Vol. 33, pp. 39-46.

Gilbert, J. J. (1970). "Monoxenic Cultivation of the Rotifer *Brachionus calyciflorus* in a Defined Medium", in *Oecologia.* Vol. 4, pp. 89-101.

Halbach, U. (1970). "Einfluss der Temperatur auf die Populationsdynamik des Planktischen Rädertiere *Brachionus calyciflorus* Pallas", in *Oecologia.* Vol. 4, pp. 176-207.

Iyer, N. and Rao, T. R. (1996). "Responses of the Predatory Rotifer *Asplanchna intermedia* to prey Species Differing in Vulnerability: Laboratory and Field Studies", in *Freshwater Biol.* Vol. 36, pp. 521-534.

Joaquim-Justo, C.; Gosselain, V.; Descy, J. P. and Thome, J. P. (1995). "Relative Importance of the Trophic and Direct Pathways on PCB Contamination in the Rotifer Species *Brachionus calyciflorus* (Pallas)", in *Hydrobiologia.* Vol. 313/314: 249-257.

Koste, W. (1978). "Rotatoria. Die Radertiere Mitteleuropas", in *Gebruder.* Vol. 1 and Vol. 2. Borntraeger, Berlin.

Poole, R. W. (1974). *An Introduction to Quantitative Ecology.* McGraw-Hill, New York.

Pourriot, R. (1965). "Recherches sur l'ecologie des Rotiferes", in *Vie et Milieu* (Suppl.). Vol. 21: pp. 1-224.

Rothhaupt, K. O. (1993). "Steady-state Growth and Carbon Metabolism of *Brachionus rubens* and *B. calyciflorus*", in Walz, N (ed). *Plankton Regulation Dynamics. Experiments and Models in Rotifer Continuous Cultures. Ecological Studies..* Vol. 98, pp. 123-132. Springer Verlag, Berlin.

Sarma, S. S. S.

_____ (1991). *Global Bibliography on Rotifera.* Bioinformatics Centre, Madurai Kamaraj University, Madurai, India. Vol. 1, pp. 1-485.

_____ (1996). "Rotifer mass Culture Systems", Chapter 3. in *International Workshop on Rotifer Culture Systems.* Laboratory Manual. UNAM Campus Iztacala, México. pp. 22-27.

_____ and Rao, T. R. (1990). "Population Dynamics of *Brachionus patulus* Muller (Rotifera) in Relation to food and Temperature", in *Proc. Indian Acad. Sci.* (Anim. Sci.). Vol. 99, pp. 335-343.

_____; Iyer, N. and Dumont, H. J. (1996). "Competitive Interactions Between Herbivorous Rotifers: Importance of food Concentration and Initial Population Density", in *Hydrobiologia.* Vol. 331, pp. 1-7.

_____; Araiza, M. A. F. and Lopéz, R. J. A. (1997). "Influence of food Concentration and Inoculation Density on the Population Growth of *Brachionus calyciflorus* Pallas (Rotifera)", in *Environment & Ecology.* Vol. 15, pp. 435-441.

Snell, T. W. and Janssen, C. R. (1995). "Rotifers in Ecotoxicology: a Review", in *Hydrobiologia.* Vol. 313/314, pp. 231-247.

Starkweather, P. L. and Keller, P. E. (1983). "Utilization of Cyanobacteria by *Brachionus calyciflorus*: *Anabaena flos-aquae* (NRC-44-1) as a sole or Complementary food Source", in *Hydrobiologia.* Vol. 104, pp. 373-377.

Weithoff, G. & Walz, N. (1995). "Influence of the Filamentous Cyanobacterium *Planktothrix agardhii* on Population Growth and Reproductive Pattern of the Rotifer *Brachionus calyciflorus*", in *Hydrobiologia.* Vol. 313/314, pp. 381-386.