

Laboratory controlled production of *Brachionus patulus* Müller 1786, using three green unicellular microalgae as food.

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ABSTRACT

In this study, a laboratory controlled culture of *B. patulus* with temperatures of $25 \pm 2^\circ\text{C}$ and a pH of 7-8, in 20 L plastic beakers with 10 L of water (10 gL^{-1} salinity). Initial culture density was 63 org mL^{-1} . Four microalgae diets were supply at $500 \times 10^3 \text{ cells mL}^{-1}$ concentration: a) *C. vulgaris*, b) *Sphaerocystis* sp., c) *H. pluvialis* y d) A mix of all three microalgae (200 mL each one). Every third day, rotifers density population was count by taking a sample of 100 mL. For this sample, 10 aliquots of 1 mL taken to obtain mean values (\pm S. D.). The highest density observed between 6 to 12 culture days in every diet. *H. pluvialis* diet obtained 477 org mL^{-1} density, *Sphaerocystis* sp. with 476 org mL^{-1} . The lowest density was *C. vulgaris* with 105 org mL^{-1} . Growth tendency curves were always a sixth grade polynomial curve. The importance of this work allows the management of other microalgae in rotifer cultures, as well as laboratory controlled production cycles of twelve days, from which, it could begin to harvest biomass excess to use it as direct food, or dry it, or implement it as raw material to elaborate other commercial diets for aquatic species.

Keywords: *Brachionus patulus*, microalgae, population density, growth tendency curve.

INTRODUCTION

Starting in the year 2000 culturing aquatic species took great relevance; therefore, it has become one of the most

important activities in Mexico, both its economic and social impact in creation jobs, food production, their faster growth as well as regional development factors (SAGARPA 2014 y ESACUA 2011).

An aspect of great importance in aquaculture is the way of feeding this organisms, although currently there is available a huge variety of balanced foods. It has observed through time and in diverse investigations, that those diets do not have nutritional levels that species require for their optimal growth; another limitation that is observe in inert diets is their high acquisition cost (Castro et al. 2003). This is why investigations oriented to microorganisms as food source, are developing where massive cultures of microalgae, rotifers, copepods and cladocerans are base of commercial production (Torrentera and Tacon 1989).

In recent decades has been considered rotifers as alternative diet, cause its nutritional contribution, fast developing, and easy cultivation (Castro et al. 2003), as well as its high survival capacity in temperature and salinity wide ranges (Lubzens 1987).

Within rotifers used as live food, there is the genus *Brachionus*, which has shown the potential for maintenance and development of aquatic species, especially in larval stages (Hung 1989). Specifically *B. patulus*, is a specie considered for its massive culture and its use as live food, because presents fast maturation, short generational

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time, high fertility, and when the eggs are produced, they show a high hatching percentage (Hernández et al. 2000).

Nevertheless, due to easy rotifer production, the biggest drawback is obtaining the right food for them, especially when culture salinity stays at 10 gL^{-1} or using freshwater rotifers. Therefore, the goal of this study is compare the culture growth of rotifer *Brachionus patulus*, using different types of freshwater microalgae as diet: *Chlorella vulgaris*, *Haematococcus pluvialis* and *Sphaerocystis* sp.

MATERIAL AND METHODS

Experimental design: Twelve 20 L plastic beakers were used; three of them were allocated for each diet (Fig.1). Temperature was controlled in the environment at $25 \pm 2^\circ\text{C}$ and a pH between 7-8. Aeration maintained constant, with minimum airflow, just to maintain the medium in constant circulation. Salinity concentration was kept in 10 gL^{-1} . Initial rotifer density per beaker was 63 org mL^{-1} , obtained from Laboratory Living Food Production Universidad Autónoma Metropolitana Xochimilco. Four experimental diets were used at this experiment: a) *Chlorella vulgaris*; b) *Haematococcus*

pluvialis; c) *Sphaerocystis* sp. and d) A mix of the three microalgae in equal concentration. Every third day, rotifers from the beakers were counted to determine their population density. Culture was maintained under these conditions; up to observe organism's mortality.

Feeding: For feeding *B. patulus* it was used three unicellular green microalgae species: *C. vulgaris*, *Sphaerocystis* sp. and *H. pluvialis*. Which were cultured in 20 L plastic beakers, inoculated with a microalgae strain conserved in bacteriological agar. Microalgae beakers were fertilized with 10 mL of Triple 17 (50 g in 500 mL of water) and 5 mL of Urea (1 Kg in 4 L of water). Temperature was maintained at $19\text{-}20^\circ\text{C}$ and constant light (40 watts white light tube) and aeration. Each week beakers from microalgae cultures were unfolded for maintaining an optimal concentration of $500 - 750 \times 10^3 \text{ cells mL}^{-1}$. Every third day 600 mL were inoculated from each microalgae. For combined diet, 200 mL were taken from each one to full fill the 600 mL of supplied food. Every third day to all beakers of each experimental diet was added 1 mL of dry active yeast (10 g in 4 L of water at 90 g L^{-1} salinity).

Sampling: Every third day, 100 mL sample was taken from each beaker, 10 aliquots of 1 mL with a micropipette Bio

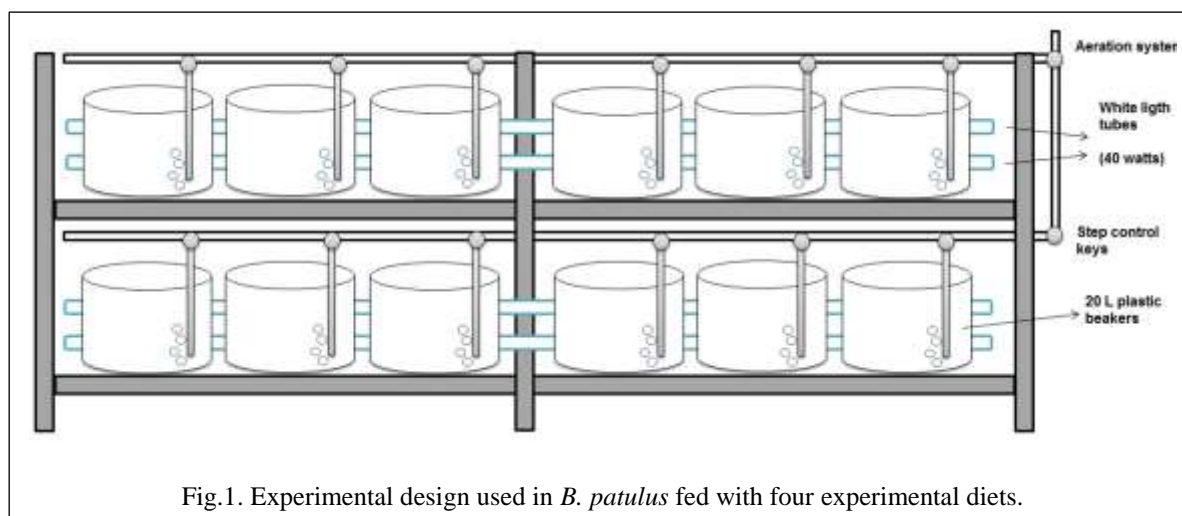


Fig.1. Experimental design used in *B. patulus* fed with four experimental diets.

Hit (1000 μ l) were taken to obtain population density average (\pm S.D.). Each aliquot was fixed with Lugol (10%) and were counted with stereoscopic microscope Olympus. Density samples were taken until rotifers population decrease until organisms die.

Data processing: A database was elaborated in Excel 2010 to determine, per sampling day, the average of organisms per milliliter. In addition, a growth tendency curve formulas were obtained from each experimental diet.

RESULTS

Mean values (\pm S.D.) of population density per experimental diet and culture day are shown in Table 1. It can observe in all cultures that *B.patulus* population decays on

21 culture days. Highest rotifers productions observed on 9 to 12 culture days. The diets with higher organism production were *Sphaerocystis* sp. and *H. pluvialis* with 476 and 477 org mL⁻¹ respectively. The lowest density was in *C. vulgaris* diet with 105 org mL⁻¹. From 15 culture days, density begins to decrease until all population dies. Culture of *B. patulus* fed with combined diet, shows at 15-21 culture days, a higher density stability.

Variance analysis letters (ANOVA) between diets per sample day show no significant differences ($P < 0.05$) (Table 1), while ANOVA analysis between samples per diet are shown at Fig.2.

Population growth tendency curves, are shown at Fig.3. In all diets, the growth formula was a sixth grade polynomial curve.

DISCUSSION

Table 1. Mean values (\pm S.D.) of *B. patulus* density (org mL⁻¹) per culture day, fed with four experimental diets.

Sampling culture days	Diet			
	<i>Cholrella vulgaris</i>	<i>Sphaerocystis</i> sp.	<i>Haematococcus pluvialis</i>	Combined
0	63 ^a	63 ^a	63 ^a	63 ^a
	± 4	± 4	± 4	± 4
3	66 ^{a,b}	73 ^d	74 ^{b,c,d}	61 ^{a,c}
	± 4	± 37	± 24	± 22
6	88 ^a	98 ^a	110 ^a	137 ^a
	± 12	± 14	± 76	± 64
9	105	424	438	352
	± 17	± 50	± 13	± 38
12	74	476 ^a	477 ^a	346
	± 13	± 10	± 18	± 45
15	45	185 ^a	179	204 ^a
	± 11	± 28	± 29	± 50
18	98 ^{a,b}	84 ^b	103 ^a	314
	± 18	± 26	± 17	± 35
21	60	93	123	173
	± 16	± 12	± 19	± 22

Same letter in a row do not show significant differences ($P > 0.05$).

The increase in density of *B. patulus* observed in this experiment using microalgae *C. vulgaris* was 166% at nine culture days over initial density; while *Sphaerocystis* sp. diet was 755% with respect to the initial and 757% with *H. pluvialis*, both at 12 culture days. Regarding to combine diet, it reached an increase of 549% with respect initial density, also at 12 culture days, obtaining densities between 346-477 org mL⁻¹, while *C. vulgaris*

days, obtained 109 ±26 org mL⁻¹ with microalgae concentration of 100 x 10³ cell mL⁻¹; while at microalgae concentration of 300 x 10³ cell mL⁻¹, obtained 296 ±20 org mL⁻¹ density; with dry yeast diet a density between 50-97 rotifers mL⁻¹ and combined diet a 251-259 rotifers mL⁻¹ density. These density differences respect this study, is mainly due to used microalgae concentration (500 x 10³ cell mL⁻¹), because difference is

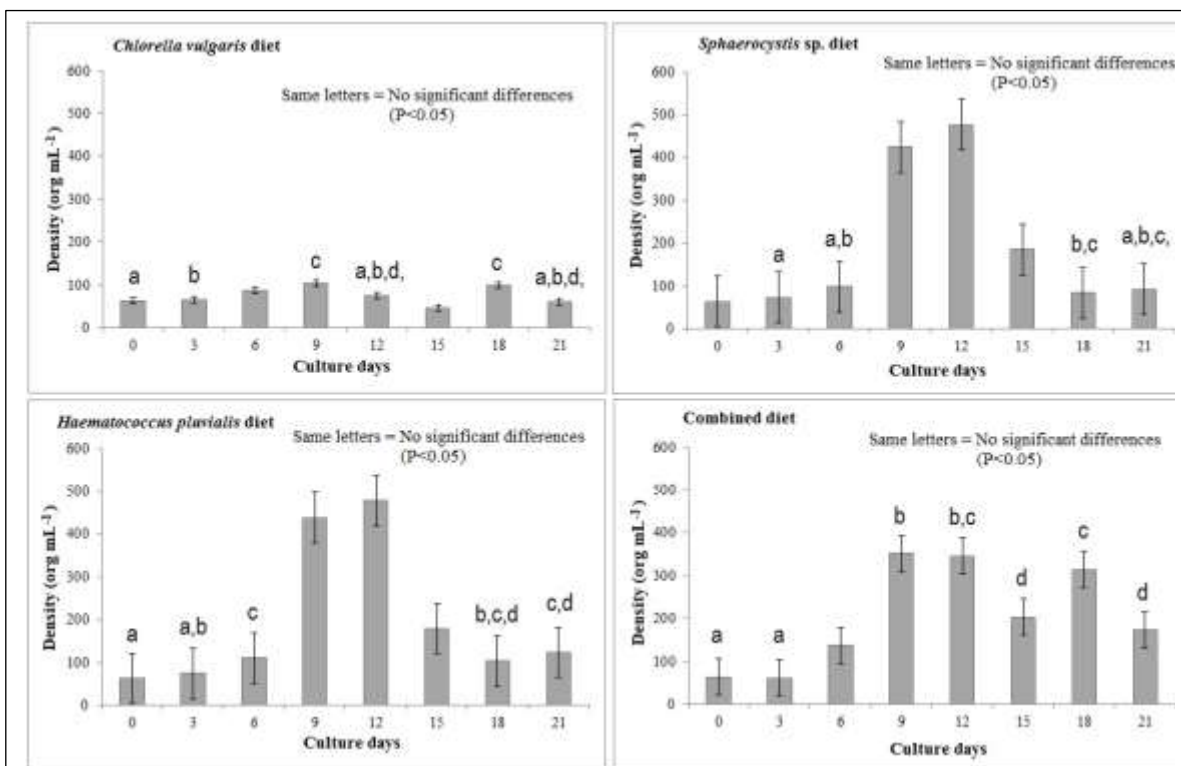


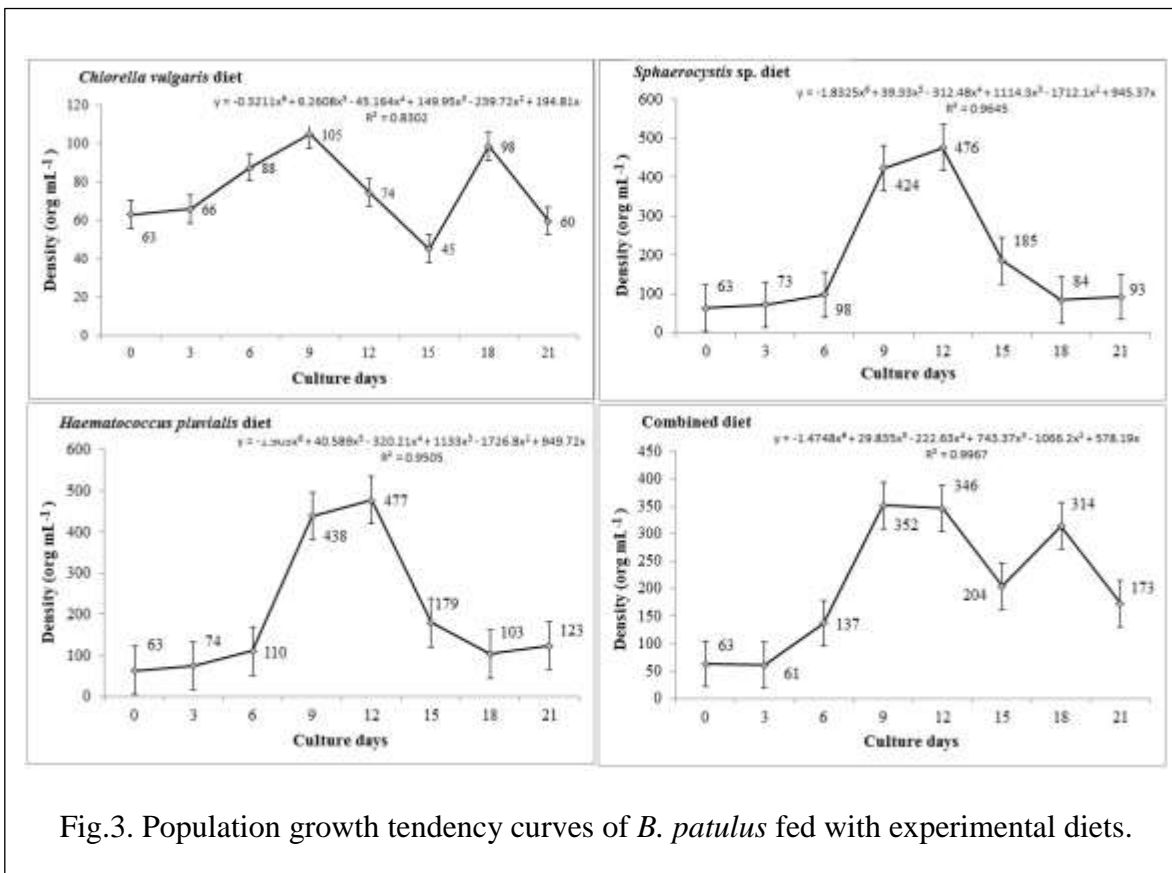
Fig.2. Analysis of variance (ANOVA) between sample days, per each experimental diets.

obtained densities between 98-105 org mL⁻¹ at 21 culture days. This obtained densities of *B. patulus* for this experiment and diets are above densities obtained by other authors.

Sarma et al. (2001a) working with *B. patulus* fed with microalgae at two different concentrations (100 x 10³ and 300 x 10³ cell mL⁻¹), with dry yeast and a combination of both diets obtained densities below what was founded at this experiment. In 14 culture

200-400 x 10³ cell mL⁻¹ more.

Sarma et al. (2001b) who studied *B. patulus* fed with *Chlorella* sp. at two different concentrations (5 x 10⁵ and 15 x 10⁵ cell mL⁻¹), found that at lower microalgae concentration obtained higher rotifer density (400 org mL⁻¹) than at higher microalgae concentration (200 org mL⁻¹). Similar densities obtained in this study with *Chlorella* sp. but below density values, respect to



the other two microalgae has and combined diet.

Pedro-Alvarez et al. (2003), worked with *B. patulus* at 1-5 g L⁻¹ salinity, fed with microalgae *C. vulgaris* at 45 x 10⁵ cell mL⁻¹ concentration, obtaining closer values to this investigation with a maximum density of 397 ± 7 org mL⁻¹ (72 org mL⁻¹ less). These authors obtained maximum density peak at 11 culture days, at difference to 12 culture days at this work. Flores-Burgos et al. (2003), which worked with *B. patulus* and *B. calyciflorus* fed with a 100% *C. vulgaris* diet; 100% *Scenedesmus acutus* and 50:50% diet of the previous ones, obtaining densities of 100-125 org mL⁻¹ with *C. vulgaris*, 60 org mL⁻¹ with *S. acutus* and with 50:50% diet 100 org mL⁻¹. Similar values to those obtained in *C. vulgaris* in this experiment, but below to obtained densities with the other two microalgae and

even with the combined diet, which were above 300%.

Sarma et al. (2007) worked *B. patulus* in 20 culture days, fed with *C. vulgaris* at two concentrations (0.5 and 1.5 x 10⁶ cell mL⁻¹). At these two concentrations, they obtained densities of 40 y 100 org mL⁻¹ respectively. These authors mentioned that a higher cellular concentration is obtained a higher rotifers density, although this isn't shown in this investigation because increasing the concentration to 5 x 10⁶ cell mL⁻¹ is obtained 105 org mL⁻¹ in *C. vulgaris* diet. This confirms with what was founded by Rueda (1993), which mentions that rotifers density don't increase or it's few at higher concentrations of *C. vulgaris*, attributing this phenomenon that organism reach maximum microalgae ingestion rate; therefore, the organism stop consuming them and

consequently its density decreases. Authors like Hirayama (1987) and Maruyama (1997), mentions that rotifer populations feeded with *C. vulgaris* show an instability, because this microalgae has a Vitamin B₁₂ deficiency which is essential to rotifers culture. This vitamin B₁₂, is founded at microalgae *H. pluvialis* (Martínez 2010), which showed maximum density of 477 org mL⁻¹. This also must be happening with microalgae *Sphaerocystis* sp. with a maximum culture density peak of 476 org mL⁻¹.

The density stability of *B. patulus* observed in culture beakers, fed with mixed diets, confirms the studies by Snell et al. (1983), and Snell and Carrillo (1984). These authors attributing this condition to inert diet supply (Salvatore and Mazzola 1981) which cover the rotifer nutritional requirements and therefore, a better wellbeing in the culture and maintain densities between 100-300 org mL⁻¹ even 21 culture days.

CONCLUSIONS

Microalgae *Sphaerocystis* sp and *H. pluvialis*, allow obtain higher rotifer densities than *C. vulgaris* diet. Also, the combined diet, allow a higher culture stability in relation to density, with a higher culture period (21 days) and not just 12 culture days with the other two microalgae's or just nine days with *C. vulgaris*.

BIBLIOGRAPHY

- Castro BT, De Lara AR, Castro MG, Castro MJ, Malpica SA. (2003). Alimento vivo en acuicultura. Depto. El Hombre y su ambiente. División de CBS. UAM. Unidad Xochimilco. 27 – 33 p.
- ESACUA (2011). Informe de Acuicultura Continental, Asociación Española de Productores de Acuicultura Continental. Unión Europea. España. 43 p. Consulta en: <http://esacua.com/web/wp-content/uploads/2011/03/2010.informe.acuicultura.pdf>.
- Espinosa-Rodríguez CA, Rivera-De la Parra L, Sarma SSS, Nandini S. (2014). Effect of food density of *Pseudokirchneriella subcapitata* and *Chlorella vulgaris* on the population growth rates of four brachionid rotifers. *Inland Waters* 4: 271-278.
- Flores-Burgos F, Sarma SSS, Nandini S. (2003). Population Growth of Zooplankton (Rotifers and Cladocerans) Fed *Chlorella vulgaris* and *Scenedesmus acutus* in Different Proportions. *Acta hydrochim. hydrobiol.* 31(3): 240–248.
- Hernández P, Martínez M, Prieto G. (2000). Aspectos reproductivos del rotífero (*Brachionus patulus* Müller 1786), en condiciones de laboratorio. Universidad de Córdoba, Facultad de Medicina Veterinaria y Zootecnia. Departamento de acuicultura 5 (2): 11.
- Hirayama K. (1987). A consideration of why mass culture of the rotifer *Brachionus plicatilis* with baker's yeast is unstable. *Developments in Hydrobiology* 42: 269-270.
- Hung M. (1989). Ensayo de cultivo de una cepa de rotífero *Brachionus plicatilis* aislada en Venezuela. *Rev. Latinoamericana de Acuicultura* 40:83-112.
- Lubzens E. (1987). Raising rotifers for use in aquaculture. *Hydrobiologia* 147:245-255.
- Martinez SA. (2010). Evaluacion del crecimiento celular y de los pigmentos obtenidos de la microalga *Haematococcus pluvialis* (Chlorophyta:Volcanes) cultivada en diferentes medios. Doctorado en tecnología avanzada. Instituto Politécnico Nacional. Área de Alimentos. México, D.F. 91 p.
- Maruyama I, Nakao T, Shigeno I, Ando Y, Hirayama K. (1997). Application of unicellular algae *Chlorella vulgaris* for the mass culture of marine rotifer *Brachionus*. *Hydrobiologia* 358: 133-138.
- Peredo-Álvarez VM, Sarma SSS, Nandini S. (2003). Combined effect of concentrations of algal food (*Chlorella vulgaris*) and salt (sodium chloride) on the population growth of *Brachionus calyciflorus* and *Brachionus patulus* (Rotifera). *Rev. Biol. Trop.* 51(2): 399-408.
- SAGARPA (2014). Desarrollo Tecnológico: Vinculación Productiva para el Desarrollo Tecnológico. La importancia de la

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- tecnología acuícola. Comisión Nacional De Acuicultura Y Pesca (CONAPESCA), MÉXICO. Consulta en: http://www.conapesca.sagarpa.gob.mx/wb/cona/desarrollo_tecnologico.
- Salvatore G, Mazzola A. (1981). Mass culture of *Brachionus plicatilis* with one integrated system of *Tetraselmis suecica* y *Saccharomyces cerevisiae*. J. World Maricul. Soc. 12(2): 61-62.
- Sarma SSS, Larios-Jurado PS, Nandini S. (2001a). Effect of three food types on the population growth of *Brachionus calyciflorus* and *Brachionus patulus* (Rotifera: Brachionidae). Rev. Biol. Trop. 49(1): 77-84.
- Sarma SSS, Nandini S, Ramírez- Pérez T. (2001b). Combined Effects of Mercury and Algal Food Density on the Population Dynamics of *Brachionus patulus* (Rotifera). Bull. Environ. Contam. Toxicol. (2001) 67:841–847.
- Sarma SSS, Rivera SA, Elizalde FH, Nandini S. (2007). Combined Influence of Food Level and Inoculation Density on Competition between *Anuraeopsis fissa* and *Brachionus patulus* or *Brachionus macracanthus* (Rotifera: Brachionidae). Russian Journal of Ecology 38 (5): 353–362.
- Snell T, Carrillo K. (1984). Body size variation among strain of rotifer *Brachionus plicatilis*. Aquaculture 37:359-367.
- Snell TW, Bieberich CJ, Fuer R. (1983). The effects of green and blue-green alga diets on the reproductive rate of the rotifer *Brachionus plicatilis*. Aquaculture 31:21-30.
- Torrentera L, Tacon A. (1989). La producción de alimento vivo y su importancia en acuicultura. Documento de campo N° 12. Proyecto Aquila. FAO. 89 p.