

ARTICLE

Pilot-scale production of the rotifer *Brachionus* sp. under different culture systems

Producción piloto del rotífero *Brachionus* sp. bajo diferentes sistemas de cultivo

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Resumen. Pequeños *Brachionus* sp. (130-230 μm) en 2011-2012 y superpequeños *Brachionus* sp. (110-140 μm) en 2013-2014, se cultivaron en un sistema por lotes (ciclos de 3 o 4 días) y en sistemas semi-continuos. Para la alimentación de rotíferos se usaron microalga concentrada (*Nannochloropsis* sp.), dosificada continuamente con una bomba peristáltica. Las condiciones del cultivo fueron provistas con aire, oxígeno e hidroximetanosulfonato de sodio. La producción total promedio por día fue 552, 602 y 459 millones de rotíferos d^{-1} , con una densidad final promedio de cosecha de 1.099 rotíferos mL^{-1} , 1.052 rotíferos mL^{-1} y 1.015 rotíferos mL^{-1} , para tres días (3-d), cuatro días (4-d) del sistema de producción por lotes y el sistema de producción semi-continuo, respectivamente. Los valores promedios producidos de rotíferos fueron adecuados para suplir los requerimientos de rotíferos en los ciclos de cultivos piloto, para producir $169 \times 10^3/\text{año}$ (2012) a 564×10^3 (2013) juveniles/año con una demanda media anual de $83,9 \times 10^6$ rotíferos/1000 juveniles producidos. Los costos totales operacionales por millón de rotíferos/día fueron bajos con el sistema de cultivo semi-continuo (\$0,23), seguido por el sistema por lotes de 4-d (\$0,55) y el sistema por lotes de 3-d (\$0,59). Estos costos de producción fueron más bajos que otros reportes de producción con alimento artificial y sistemas de recirculación. Los principales componentes de los costos operacionales fueron alimento (71-77%) y personal (7-11%). La mejor estabilidad y confiabilidad de la producción fue con el sistema semi-continuo, el cual garantizó las cantidades requeridas diariamente para los cultivos de cría larval más altos. Para posibles mejoras y aumento de la producción, la discusión se basa en términos de eficiencia financiera.

Palabras clave: Sistema de cultivo por lotes, sistema de cultivo semi-continuo, análisis de costos operacionales, larva de peces marinos

Abstract. Small *Brachionus* sp. (130-230 μm) in 2011-2012 and tiny *Brachionus* sp. (110-140 μm) in 2013-2014 were reared in batch culture (3 or 4 day cycles) and semi-continuous systems. For feeding rotifers, nonviable microalgae (*Nannochloropsis* sp.) were used and were continuously dosed with peristaltic pumps. The cultures provided air, oxygen, and sodium hydroxymethanesulfonate. An average total daily production of 552, 602 and 459 million rotifers d^{-1} with mean final densities of 1,099 rotifers mL^{-1} , 1,052 rotifers mL^{-1} and 1,015 rotifers mL^{-1} were harvested in three day (3-d) and four day (4-d) batch culture systems and semi-continuous culture systems, respectively. The average values of rotifers produced were adequate to supply the rotifers required in the parallel pilot rearing larval cycles, and $169 \times 10^3/\text{yr}$ (2012) to 564×10^3 (2013) juveniles/yr were produced with a mean annual demand of 83.9×10^6 rotifers/1,000 juveniles produced. The total operational cost per million rotifers/day was lower for the semi-continuous culture system (\$0.23), followed by the 4-d batch system (\$0.55) and 3-d batch system (\$0.59). These production costs were lower than those of other reports with artificial feeds and recirculation systems. The main components of the total operational cost was food (71-77%) and labor (7-11%). The best production stability and reliability were in the semi-continuous system, which best met the required daily quantities for the larval rearing trials. For possible improvements and increased production, the results are discussed in terms of financial efficiency.

Key words: Batch culture system, semi-continuous culture system, operational cost analysis, marine fish larvae

INTRODUCTION

There are no efficient dried diets for the initial feeding of marine and estuarine fish larvae. Often, the larvae have small mouths; therefore, growers use small rotifers as the first feed (Su *et al.* 1994, Duray *et al.* 1996, Leu *et al.* 2003). Thus, marine-

estuarine pilot and commercial scale hatcheries need to produce significant quantities of high quality rotifers at the right time. The challenge is meeting the larval rearing demands with longstanding, flexible and reliable production systems and

acceptable financial efficiency costs (Hagiwara *et al.* 2001). This is essential for juvenile mass production stability.

The first system developed in the 60s was a semi-intensive batch culture system that reached ~ 200 rotifers mL⁻¹ (Hirata 1980, Lubzens 1987) and was the basis for the semi-intensive and semi-continuous system, which can produce up to 300 rotifers mL⁻¹ (Fukusho 1989). In more recent years, full-grown intensive and semi-continuous systems that can reach densities of ~ 7,000 to 30,000 rotifers mL⁻¹ have been used (Yoshimura *et al.* 1997, Suantika *et al.* 2001, 2003; Rombaut *et al.* 2003, Bentley *et al.* 2008), some with a much higher density of 160,000 rotifers mL⁻¹ (Yoshimura *et al.* 2003).

Batch culture systems require several tanks that are harvested at certain time intervals and require increased space and labor, and the results can be unstable and unpredictable (Dhert *et al.* 2001). One of the important causes of instability of the batch culture system is the water quality of the culture, together with pathogenic bacteria present in the tanks (Dhert *et al.* 2001, Rombaut *et al.* 2001). Water quality is improved with semi-continuous systems using microalgae and yeast, without water flow (Lubzens 1987), and the systems are further improved with recirculation (Suantika *et al.* 2003).

The tendency of marine and estuarine larval rearing protocols is toward intensive culture; therefore, the demand for rotifers has also increased. For example, the production of one ton (fresh weight) of rotifers (~ 400 x 10⁹ rotifers) is required to produce 10 million juveniles of the gilthead seabream (*Sparus aurata*) in the Mediterranean region (Zmora *et al.* 1991). In this context, we designed a pilot-scale plant for research and technology development regarding marine and estuarine juvenile mass production (Alvarez-Lajonchère *et al.* 2007). The plant was built at the Mazatlan unit of the Centro de Investigación en Alimentación y Desarrollo (CIAD-Mazatlan), with an estimated maximum daily rotifer production of 2.5 x 10⁹ (Alvarez-Lajonchère *et al.* 2007). An intensive batch system was the first culture adopted, using dry diets (Alvarez-Lajonchère & Sánchez-Téllez 2013); the system was evaluated in a single trial.

The object of the present study was to assess the efficiency and operational costs of three new small rotifer production systems in the plant using nonviable microalgae (*Nannochloropsis* sp.): a 1) three day (3-d) batch culture, 2) four day (4-d) batch culture and 3) semi-continuous culture system. With this design, attempts were made to avoid the microalgae production facility and pollution in the rearing environment by dry diets, working without water flow or recirculation. The goal was to select a system that could reach at least 1,000 rotifers mL⁻¹ or higher in 1,000-L tanks to feed

the daily demands of spotted rose snapper (*Lutjanus guttatus*) pilot-scale larval rearing with production stability, reliability and low operational costs.

MATERIALS AND METHODS

This research was conducted in the marine and estuarine fish pilot-scale plant at the Mazatlan unit (CIAD-Mazatlan) in parallel with spotted rose snapper juvenile production cycles between 2011 and 2014.

ROTIFER STRAINS

The research experiments used two small *Brachionus* sp. rotifers strains. First, during 2011 and 2012, the cultured strain was a small one (130-230 µm lorica length) used in the earlier work in the pilot-scale plant. Second, during 2013 and 2014, the technicians of the pilot-scale plant isolated a smaller strain (110-140 µm) from the mud bottom. The maintenance system for both strains followed Velasco-Blanco *et al.* (2011).

CULTURE SYSTEMS

Two different phases for the experiments were defined. The first phase was to set up a semi-automatic batch culture system to reach 900-1,000 rotifers mL⁻¹. In the second phase, a semi-automated and semi-continuous system was developed, reaching 1,000-1,500 rotifers mL⁻¹ with daily harvests between 10 and 25% of the culture volume, without flow or recirculation in the systems. Nonviable microalgae (*Nannochloropsis* sp.) were used to feed rotifers. Table 1 describes the main facilities and equipment used according to their use in each system. During 2011, the batch culture systems were used. Rotifer rearing took place in 1,200-L white fiberglass cylindroconical tanks supplied with constant aeration, pure oxygen, and feed. The temperature of the seawater was 28 ± 2°C with a salinity of 30 ± 3, and the water was treated as described by Alvarez-Lajonchère *et al.* (2007).

The batch culture system was kept at a constant volume of 1,000-L, reaching a density of ~ 1,000 rotifers mL⁻¹ before harvest. The system ran on 3-d or 4-d cycles. One tank was harvested every day at the end of the cycle and another started the new cycle, using a portion of the harvested rotifers (~ 500 millions). The total daily harvests were divided to cover the required quantity in the spotted rose snapper larval rearing tanks and for other research experiments. The mean stocking density for the 3-d batch culture system was 570 ± 90 rotifers mL⁻¹, and 492 ± 55 rotifers mL⁻¹ were used for the 4-d batch culture system.

Table 1. Results of rotifer production with the three culture systems (mean ± sd) / Resultados de la producción de rotíferos con los tres sistemas de cultivo (promedio ± desviación estándar)

Year	No of cycles	Days per cycle	Mean daily density (rotifer/mL)	Fertility (%)	Total rotifer harvested (x 10 ⁶)	Mean rotifer harvested (x 10 ⁶)	Rotifer used for larval rearing (x 10 ⁶)	Juveniles produced	Rotifers per 1 000 juveniles (x 10 ⁶)
A) 2011*	45	3	1,163.0 ± 252.3	24.0 ± 7.90	24,838	552	16,419	31,954	513.83
B) 2011*	35	4	1,128.0 ± 118.7	19.9±7.5	21,071	602	14,796	40,326	366.91
C) 2011**	3	10	866.0 ± 214.92	20.7±9.9	5,880	196	10,674	96,707	110.37
2012**	3	24	882.6 ± 138.47	1.6±5.9	15,373	167	15,373	169,825	90.52
2013**	2	50	931.8 ± 100.00	18.4±6.8	45,928	459	45,928	563,562	81.50
2014**	2	50	1,014.9 ± 148.36	17.9±7.0	31,875	318	31,875	309,883	102.86

*Batch culture system, **Semi-continuous system

NOTE: Rotifers used for larval rearing (x 10⁶) in cycle C) 2011, were complemented with the production from cycle B) 2011

During the 2011 and 2012 seasons, a nonviable *Nannochloropsis* sp. was used to feed the rotifers (Rotigrow plus[®] from Reed Mariculture Inc., USA). The supply rate was 1.5 to 2.0 mL/million rotifers/day, according to the provider recommendations; the feed was supplied with peristaltic pumps (Uni-dose model U041-281TT, ~ 5 L/h) (De Wolf *et al.* 1998). Pure oxygen was supplied from a Dewar 130 m³ tank. Organic matter was collected with two devices, a sphere within the water column and another at the water surface with an air-lift system (Schipp *et al.* 2007). Sodium hydroxymethanesulfonate (ClorAm-X[®], AquaScience Research Group, Inc., USA) was supplied according to Riche *et al.* (2006) and Bentley *et al.* (2008).

For the semi-automated and semi-continuous systems tested in the present study, harvests were performed daily, allowing for partial extraction of the culture media, which was replaced with new water (Fukusho 1989). The extracted volume (~ 10-25%) depended on the rotifer reproduction rate (% fertility) and demand of the larval rearing tanks. The stocking density was, in 2013, 922.7 ± 86.06, and in 2014, 1,037 ± 109.01 rotifers mL⁻¹ in 1,000-L. This system was tested in 10-d, 24-d, and 50-d of continuous culture.

For this semi-continuous system new equipment and materials were used, in addition to those used with the batch culture systems (Table 1). This included a new oxygen supply (Respironics Milenium, Philips Model M10, with a capacity of 10 L/min.), ultra-filter equipment 0.2 µm (FreshPoint[™] U440 Pentek) and calorimetric pump for temperature control of the rearing room (Fan & Coil, Heat Pump, model 4TWR592e with

a cooling capacity of 24,000 BTU and a heat pump, model MWD 516 D1, with a nominal capacity of 2 tons). Rotigrow plus[®] was replaced with Nano 3600[™] (Reed Mariculture Inc., USA). The supply rate was 1.5 mL/million rotifers/day, according to the provider recommendations. Rectangular Scotch-Brite[®] fibers were submerged as a new organic matter traps (Lubzens *et al.* 2001).

DAILY GROWTH

Before the daily harvest, the rotifer density in each tank was estimated, taking three 0.5-mL subsamples from a first sample of 200 mL using a micropipette (Transferpette brand, 100-1,000 µL). In a Sedgewick Rafter counting chamber, the live rotifers were observed, and they were counted, after fixation with two drops of lugol solution, under a microscope (Leica DM500, EZ Tube[™]). The rotifers were not counted when they had empty and transparent lorica.

SPECIFIC GROWTH RATE

The Specific Growth Rate (SGR) from 3-d and 4-d batch culture systems was estimated by the following equation:

$$SGR = \frac{\ln N_t - \ln N_0}{t}$$

where SGR= specific growth rate, N_t= rotifer concentration at time *t*, N₀= rotifer concentration at time 0 (stocking) and *t*= days of culture.

FERTILITY

Fertility (F) was estimated daily, with the same samples used for rotifer culture growth estimation, by the following formula:

$$F = \frac{N_t}{N} \times 100$$

where F= fertility (%), N_t= rotifers with at least one egg, N= total number of rotifers

ECONOMIC AND FINANCIAL ANALYSIS

Overall calculations were carried out in US dollars (\$, USD), with an exchange rate MXN/USD of \$18.00. Capital costs were different according to the culture system (Table 1). Fixed and variable costs were considered operational costs. The main fixed costs were the annual depreciation of buildings and equipment, estimated according to their useful life and use for rotifer production. The water (seawater and freshwater) volumes and air consumed during rotifer production were estimated according to Ibarra-Castro *et al.* (2013). Among the variable costs in each system, labor, feed, oxygen, air, electricity and consumables were considered on a monthly basis (Table 2).

Labor was calculated according to the required working hours at a rate of \$1.61/h.

ENVIRONMENTAL PARAMETERS

Every day at 0800 and 1800 h, the dissolved oxygen concentration (DO, mg L⁻¹), percentage of oxygen saturation (%), pH, and temperature (°C) were measured with Hach multiparameter equipment (HQ30d, HQd™), while salinity was measured with a refractometer RHS-10ATC. Ammonia was neutralized with ClorAm-X® (Riche *et al.* 2006, Bentley *et al.* 2008).

STATISTICAL ANALYSIS

The normality and homogeneity of variance for the rotifer density and SGR in batch systems was determined by Shapiro Wilk and Kolmogorov-Smirnov tests, respectively. If data met the requirements for a parametric variance analysis, one-way ANOVA was carried out (Zar 2010). Statistical analyses were performed using the SigmaPlot v11 software (©Systat Software Inc.).

Table 2. Estimated capital investment for rotifer (*Brachionus* sp.) in three-day (3-d, year 2011) and four-day (4-d, year 2011) batch systems and a semi-continuous system, according to pilot-scale utilization and useful life / Estimación del capital de inversión para la producción de rotíferos (*Brachionus* sp.) en sistema por lotes de tres días (3-d año 2011), sistema por lotes de cuatro días (4-d, año 2011) y sistema semi-continuo, acorde a su utilización en la escala piloto y vida útil

Facility	Item	3-d batch system			4-d batch system			Semi-continuous system		
		Quantity	Annual cost	Utilization cost	Quantity	Annual cost	Utilization cost	Quantity	Annual cost	Utilization cost
Buildings	Seawater intake system	1	4,058	10.1	1	5,410	13.52	1	1,352	3.40
	Small inoculum room	10 m ²	25	4	10 m ²	25	4	10 m ²	25	4
	Rotifer production room	30 m ²	25	4	30 m ²	100	15	10 m ²	15	3
Other installations	Seawater treatment room	1	30	4.50	1	40	6	1	21	3
	Blower room	1	11.4	2	1	11.4	2	1	11.4	2
Equipment	Seawater pump	1	400	100	1	500	125	1	300	75
	Temperature control system	-	-	-	-	-	-	1	167	167
	Seawater treatment and pipework installations	1	2,183	546	1	2,910	728	1	728	182
	Ultra-filter	-	-	-	-	-	-	1	222	222
	Freshwater treatment and pipework installations	1	649	162	1	865	216	1	216	54
	Blower	1	1,750	210	1	2,333	280	1	583	70
	Air pipework installations		300	75		400	100	1	150	38
	Oxygen system (Dewar tank)	1	111	111	1	167	167	-	-	-
	Oxygen concentrator	-	-	-	-	-	-	1	167	167
	Laboratory equipment	several	510	255	several	510	255	several	510	255
	Peristaltic pump	3	180	180	4	240	240	1	60	60
	Tanks	1,2 m ³ tank	3	90	90	4	120	80	1	30
300-L tank		1	45	45	1	45	45	1	45	45
100-L tank		1	30	30	1	30	30	1	30	30
Total capital cost			10,397			13,706			4,632	
Total annual depreciation				1,828.6			2,307			1,410

RESULTS

The first phase was to set up a semi-automatized batch culture system. Forty-five cycles were carried out with the 3-d system during 2011, with a total harvest of $16,419 \times 10^6$ rotifers (mean of 552 million/day) (Fig. 1A). Thirty-five cycles were carried out with the 4-d system, with a total harvest of $14,796 \times 10^6$ rotifers (mean of 602 million rotifers/day) (Fig. 1B). The maximum and mean final densities with the 3-d batch system were $1,600$ rotifers mL^{-1} and $1,099$ rotifers mL^{-1} , respectively. By contrast, $1,437$ rotifers mL^{-1} and $1,052$ rotifers mL^{-1} maximum and mean final densities were obtained with the 4-d batch system. From the rotifers produced with the batch systems, a total of 31,215 million were supplied to the larval rearing tanks of the spotted rose snapper, which produced 72,280 45-d juveniles (Table 1).

In the second phase, the semi-automated and semi-continuous system was tested. The semi-continuous system allowed high production stability (Fig. 2). After the initial results with 10-d cycles in 2011 and 24-d cycles in 2012, all other production cycles were carried out for a duration of 50-d in 2013 and 2014. The mean daily production reached 459.3 million in 2013 and 318.8 million in 2014. The mean densities were 931.8 (2013) and 1,014.9 (2014) rotifers mL^{-1} . The highest density achieved in both years was 1,120 rotifers mL^{-1} .

The 45-d juveniles produced were 563,562 in 2013 and 309,883 in 2014 (Table 1). All rotifers produced with the semi-continuous system were used to feed rose spotted snapper larvae, with a total of 1,130 million juveniles produced during those years, fed with a mean of 83.9 million rotifers per 1,000 juveniles (Table 1).

GROWTH

Rotifer culture growth per day in the 3-d and 4-d batch systems showed a linear tendency to increase density independent of the duration of the rearing cycle (Fig. 3). Although the mean stocking density of the 3-d system was higher than the 4-d system, there was no significant difference ($F_{(1,79)} = 13.33$, $P > 0.05$). On the third day of culture, the mean density of the 3-d system was $1,104 \pm 223$ rotifers mL^{-1} , which was significantly higher ($F_{(1,79)} = 31.38$, $P < 0.05$) than the mean density of the 4-d system (873 ± 127 rotifers mL^{-1}).

The growth in the semi-continuous system compared with the batch culture system, and due to the different nature of the systems, can only be observed in the daily growth of the culture submitted to daily harvests to cover the demands of the larval rearing tanks (Fig. 2). However, in the 50-d cycles, the density started to decrease and show less stability at end of the third week or at the fourth week (Fig. 2).

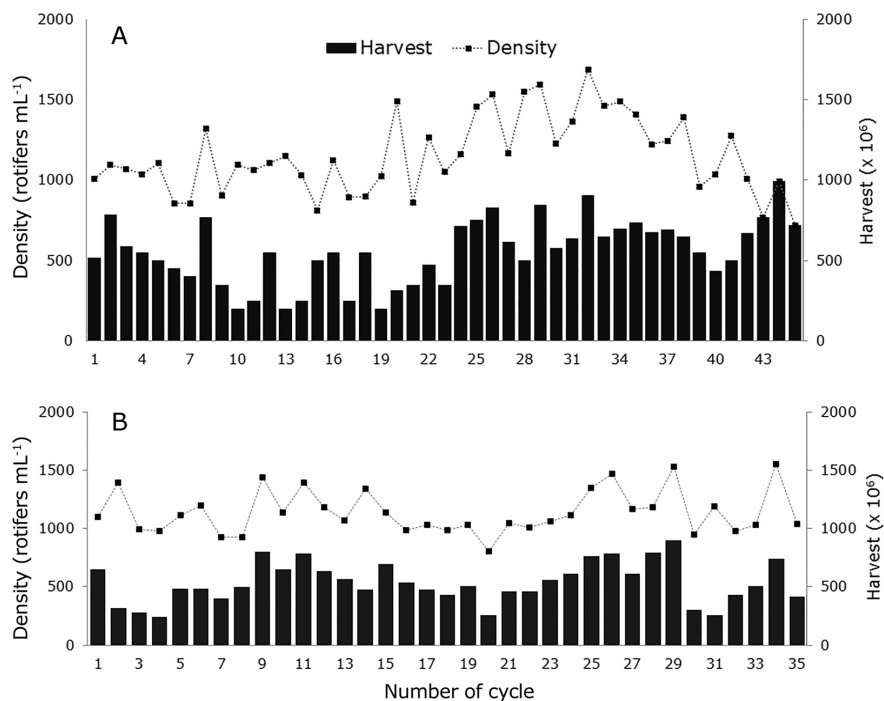


Figure 1. Mean final density per cycle of *Brachionus* sp. produced in 1,000 L tanks and daily rotifer harvests to supply spotted rose snapper *Lutjanus guttatus* larvae: A) with the 3-d batch system; B) with the 4-d batch system / Densidad promedio final por ciclo de rotíferos *Brachionus* sp., producidos en tanques de 1.000 L y rotíferos cosechados diariamente para suplir las necesidades del cultivo larvario del pargo flamenco *Lutjanus guttatus*: A) con el sistema de cultivo por lotes de 3-d; B) con el sistema de cultivo por lotes de 4-d

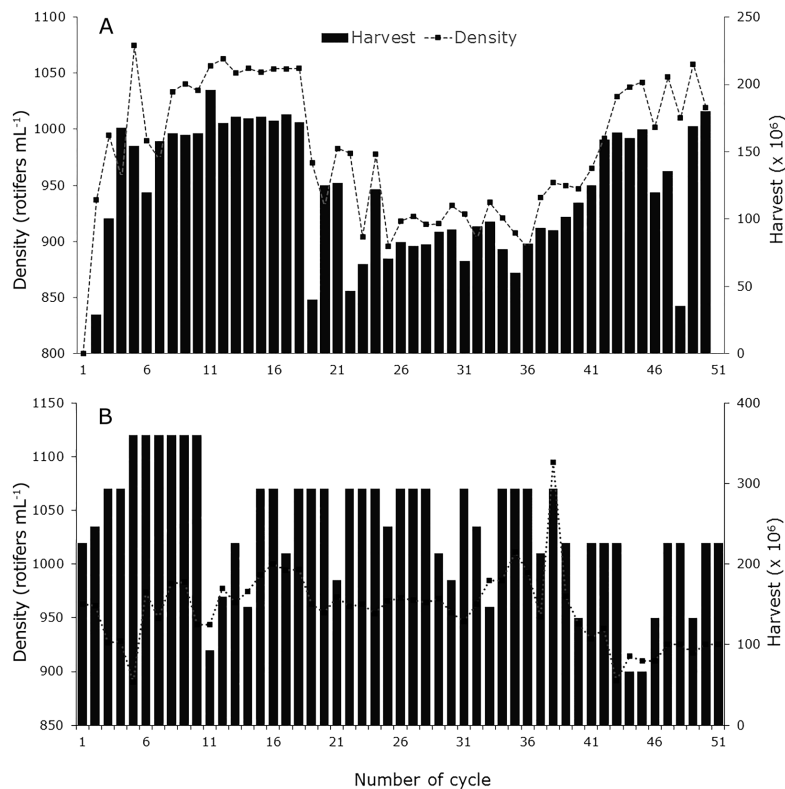


Figure 2. Mean final density per cycle of *Brachionus* sp. produced with the semi-continuous system in 1,000 L tanks, and the daily rotifer harvests to supply spotted rose snapper *Lutjanus guttatus* larvae: **A)** 2013; **B)** 2014 / Densidad promedio final por ciclo de rotíferos *Brachionus* sp., producidos con el sistema semi-continuo en tanques de 1.000 L y rotíferos cosechados diariamente para suplir las necesidades del cultivo larvario del pargo flamenco *Lutjanus guttatus*: **A)** 2013; **B)** 2014

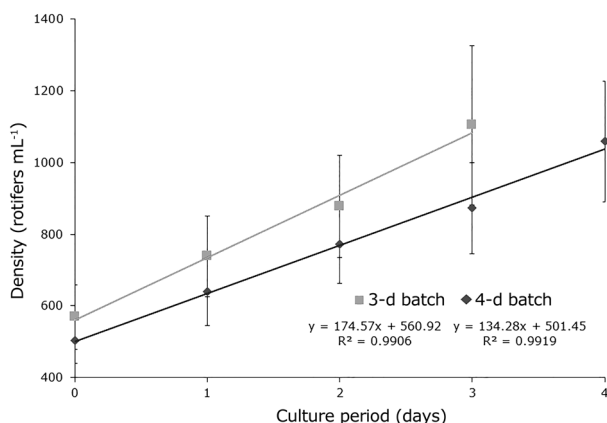


Figure 3. Mean daily growth of *Brachionus* sp. cultured for 45 cycles with the 3-d batch system and 35 cycles with the 4-d batch system / Crecimiento promedio diario del rotífero *Brachionus* sp., cultivado durante 45 ciclos con el sistema por lotes de 3-d y 35 ciclos con el sistema por lotes de 4-d

SPECIFIC GROWTH RATE

The SGR in the 3-d and 4-d batch system was from 0.12 to 0.26. The mean lowest values were observed a day before the harvest (Fig. 4). In the 3-d batch system, there was an increase from 0.17 ± 0.15 (2nd day) to 0.22 ± 0.12 (3rd day) at the harvest day. In the 4-d batch system, the SGR was 0.12 ± 0.14 (3rd day) and increased to 0.19 ± 0.12 (4th day) at the harvest day. The highest SGR mean occurred in first day of the 3-d batch system (0.26 ± 0.15). At the third day of culture, the 3-d batch system and the 4-d batch system showed significant differences ($F_{(1,79)} = 12.09, P < 0.05$).

OPERATIONAL COST ANALYSIS

Estimated annual capital costs of facilities and equipment for rotifer production were \$10,397 for the 3-d batch system, \$13,706 for the 4-d batch system, and \$4,632 for the semi-continuous system (Table 2). Total operational costs were \$9,780.27, \$9,921.41, and \$3,119.27 for the 3-d batch, 4-d batch and semi-continuous systems, respectively. In general,

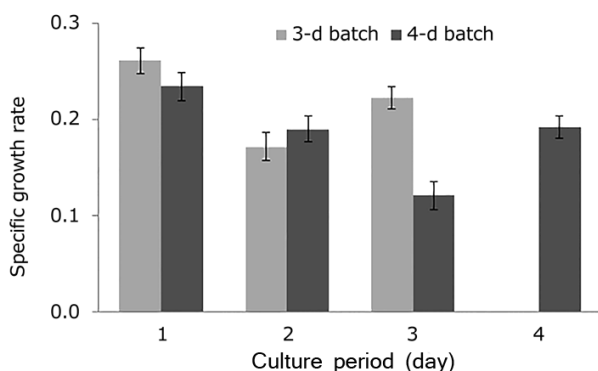


Figure 4. Mean Specific Growth Rates per day of *Brachionus* sp. for 45 cycles with the 3-d batch culture and 35 cycles with the 4-d batch culture system / Tasa de crecimiento específico promedio por día de *Brachionus* sp., para 45 ciclos con el sistema por lotes de 3-d y 35 ciclos con el sistema por lotes de 4-d

equipment was the most important capital cost, followed by buildings (Table 3). The mean monthly depreciation values were \$317.66, \$374.10, and \$153.98 for the 3-d batch, 4-d batch and semi-continuous systems, respectively. Among the operational costs, the most important was feed (70.9-76.5%), followed by labor (6.8-10.8%) (Fig. 5). The total cost/million rotifers/day were \$0.59, \$0.55, and \$0.23 for the 3-d batch, 4-d batch and semi-continuous systems, respectively (Table 4).

ENVIRONMENTAL PARAMETERS

Dissolved oxygen and oxygen saturation ranged between 8.6-8.7 ppm and 110.3-118.4% in batch systems, respectively, while it was 10-11 ppm and 115-170% in the semi-continuous system, respectively. The temperature was between 27°C and 29°C in all systems, and it was lower and more stable in the semi-continuous system. The pH was between 7.0 and 7.6 in the batch systems and oscillated approximately 7.5 in the semi-continuous system.

Table 3. Operational cost estimates (USD) by fixed and variable expenditures per month with each of the rotifer (*Brachionus* sp.) production systems: three day (3-d) batch culture (2011), four day (4-d) batch culture (2011), and a fifty day (50-d) semi-continuous system (2013) applied at a pilot-scale / Estimación de los costos operacionales (USD) de los gastos fijos y variables por mes con cada uno de los sistemas de producción de rotíferos (*Brachionus* sp.): Sistema por lotes de tres días (3-d) (2011), sistema por lotes de cuatro días (4-d) (2011) y sistema semi-continuo de 50 días (50-d) (2013), aplicados a escala piloto

Item	3-d batch	4-d batch	Semi-continuous
	45 cycles	35 cycles	Two 50-d cycles
Variable costs			
Labor	676.67	676.67	338.33
Electricity	76.00	66.33	76.78
Feeds	7,421.11	7,586.67	2,211.67
Oxygen	485.67	391.89	85.94
Aeration	2.22	1.67	1.67
Consumables	180.34	139.45	29.70
Repair and maintenance	170.00	230.00	80.00
Total variable costs	9,012.01	9,092.68	2,824.09
Fixed costs			
Depreciation			
Buildings	24.60	40.52	15.40
Equipment	112.82	151.73	102.10
Total depreciation	137.42	192.25	117.50
Administration	180.24	181.85	36.48
Total fixed costs	317.66	374.10	153.98
Contingency	450.60	454.63	141.20
Total operational costs	9,780.27	9,921.41	3,119.27
Rotifer production/day (10^6)	552	602	459
Operational costs/million rotifers/day	0.59	0.55	0.23

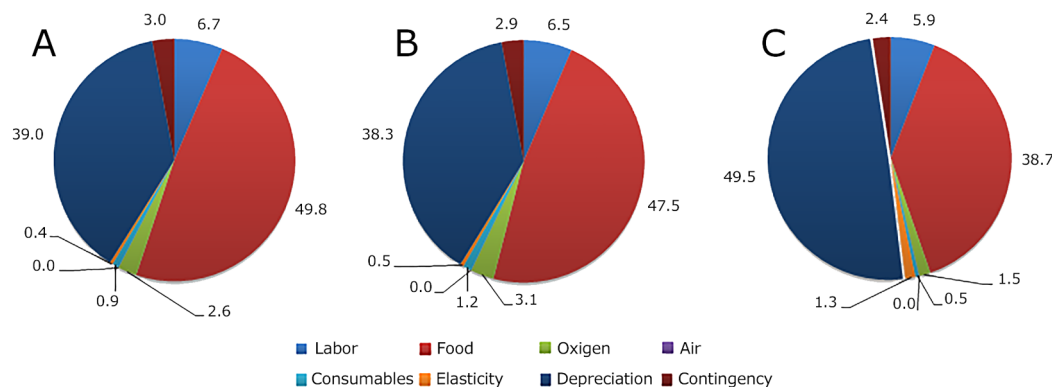


Figure 5. Estimated operational costs (%) of each *Brachionus* sp. for the culture systems: A) 3-d batch system; B) 4-d batch system; C) semi-continuous system / Costos operacionales estimados (%) para cada sistema de cultivo de rotíferos *Brachionus* sp. Sistema de cultivo: A) sistema por lotes de 3-d; B) sistema por lotes de 4-d; C) Sistema semi-continuo

Table 4. Comparison of other reports similar to the present study / Comparación de otros reportes similares al presente estudio

Items	Suantika <i>et al.</i> (2003)		Bentely <i>et al.</i> (2008)		Alvarez-Lajonchère & Sánchez-Téllez (2013)		Present study	
	Batch culture	Semi-continuous	Batch culture	Semi-continuous	Batch culture	Batch culture	Batch culture	Semi-continuous
Culture volume	n.d.	750	125	125	1,000	1,000	1,000	1,000
Culture density mL ⁻¹	860	7,000		6,500	1,595	1,099	1,052	1,015
Daily production (x 10 ⁶)	2,000	2,000	106	170	1,600	552	602	459
Feed	Culture Selco		Nonviable <i>N. oculata</i> paste (Reed Mariculture Inc.)		Culture Selco Plus®	Rotigrow plus® (Reed Mariculture, Inc.)		Nano 3600™ (Reed Mariculture Inc.)
Cycle duration	4 days	27 days	n.d.	30 days	4 days	3 days	4 days	50 days
Cost (\$, US)/10 ⁶ rotifers/day	31.69	10.39	0.46	0.29	1.65	0.59	0.55	0.23

(n.d.) No data available

DISCUSSION

In marine and estuarine fish hatcheries, it is essential to establish a stable and reliable system for the intensive mass production of rotifers to cover the daily demands of the larval tanks; this organism is the most important live feed for fish larvae (Dhert *et al.* 2001, Lubzens & Zmora 2003). Thus, it is necessary to produce rotifers with an efficient and cost-effective system, in the required quantities, of the right sizes, and of high nutritional quality for each larval rearing period (Hagiwara *et al.* 2001, Alvarez-Lajonchère & Hernández Molejón 2001).

Recently, efficient semi-continuous culture systems have been developed for rotifer production with recirculation systems. The systems are based on live and concentrated microalgae (Fu *et al.* 1997, Lubzens *et al.* 1997, Schipp *et al.* 2007) or dried feeds (Suantika *et al.* 2003) with low costs.

When the work with marine and estuarine fishes started in CIAD Mazatlan with experimental facilities, rotifers were cultured with semi-intensive techniques, using live microalgae with 3-d or 4-d batch culture systems. The yields were approximately 140 rotifers mL⁻¹ (Velasco & Duncan 2002), and the yields later increased to 334 rotifers mL⁻¹ (Guzmán 2004). Later, starting work with the pilot-scale plant (Alvarez-Lajonchère *et al.* 2007), a pilot study of intensive culture techniques allowed production yields of 1,595 rotifers mL⁻¹ with Culture Selco plus® (INVE Aquaculture Inc., USA) in 1,000 L tanks and a 4-d batch culture system made in a single cycle (Alvarez-Lajonchère & Sánchez-Téllez 2013).

In the present study, three production systems, 3-d and 4-d batch and semi-continuous culture techniques, were assessed. For the three systems, the feed source was freeze microalgae,

following Shipp *et al.* (2007), although those authors used live frozen *Chlorella* paste (Super Fresh Chlorella - V12®), which was not available to be used in the present study. A nonviable *Nannochloropsis* sp. had to be used, without the results achieved by the other product (Ibarra-Castro *et al.* not published). This could influence the results of the present study because rotifers fed with live microalgae have enhanced fertility in comparison with those fed nonviable microalgae or dried feeds.

Although the fertility obtained in this study was ~ 20% in the 3-d and 4-d batch culture systems and the semi-continuous 10-d culture, it was lower than that of Chew & Lim (2005), Alvarez-Lajonchère & Sánchez-Téllez (2013), and Jabeur *et al.* (2013). However, when the duration of the production cycles was longer (24 and 50 d) in the semi-continuous system, the fertility decreased, with mean values of ~ 15%. This does not occur with live microalgae, and this could show that the environmental conditions and feeding should be improved, as suggested by Delbos & Schwars (2008).

The fertility and SGR obtained in the present study were lower than dried feeds (Suantika *et al.* 2003, Alvarez-Lajonchère & Sánchez-Téllez 2013) or with live microalgae (Lubzens & Zmora 2003, Schipp *et al.* 2007). Yields were between 900 and 1,000 rotifers mL⁻¹, which could be considered admissible for hatcheries in tropical and subtropical regions (Moretti *et al.* 1999, Alvarez-Lajonchère & Hernández Molejón 2001, Schipp *et al.* 2007).

Yoshimura *et al.* (2003) and Alvarez-Lajonchère & Sánchez-Téllez (2013) emphasized the importance of controlling water quality. Water quality is essential to maintain high rotifer concentrations. In the present study, these factors were within the adequate range for rotifers, although the rather low fertility could be taken as an indication that some of the factors should be improved in the long cycles with the semi-continuous system.

The three production systems compared in the present study allowed the production of 1,000 rotifers mL⁻¹. The semi-continuous system had better stability, flexibility and reliability, with less labor and consequently lower production cost, in agreement with other reports (Dhert *et al.* 2001, Suantika *et al.* 2003, Bentley *et al.* 2008). Nonetheless, its density was lower than in other reports (Suantika *et al.* 2003, Bentley *et al.* 2008). The production covered the rotifer demand from parallel pilot-scale larval rearing, operated at the plant, which reached a maximum production of more than 550,000 45-d juveniles in 2013 and had higher financial benefits.

The usual intensive production systems have two main operational cost items, feed and labor, as in the present study

(Suantika *et al.* 2003). These systems are characterized by a high cost of equipment (with recirculation systems) and efficient operational costs to reach high densities of ~ 7,000-8,000 rotifers mL⁻¹ (Table 4; Suantika *et al.* 2003, Bentley *et al.* 2008), much higher than the intensive systems in the present study.

The success in intensive rotifer farming systems have been due to the availability of concentrated microalgae (Yoshimura *et al.* 1997, 2003; Schipp *et al.* 2007) and dried commercial diets such as Culture Selco®, Selco Plus® and Ori-Culture® (Suantika *et al.* 2000, 2003; Alvarez-Lajonchère & Sánchez-Téllez 2013). These products reduce the production costs of live microalgae, enhance stable and predictable rotifer culture, and provide for the needs of smaller facilities, with fewer supplies and less labor (Suantika *et al.* 2003, Alvarez-Lajonchère & Sánchez-Téllez 2013).

In conclusion, total operational cost analysis in the present study showed that the semi-continuous system was the most efficient, even more so than the dried diets tested by Suantika *et al.* (2003) and Alvarez-Lajonchère & Sánchez-Téllez (2013). The operational cost could be improved if live microalgae could be produced by a photobioreactor operating in the pilot-plant instead of importing nonviable microalgae. Further improvement could occur if the environmental parameters allowed for an increase in fertility and culture density. Other possible improvements could arise by lowering the salinity to 20-25 and using an open flow or a recirculation system.

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