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Intensive Rotifer Production in a Pilot-scale Continuous Culture Recirculating System Using Nonviable Microalgae and an Ammonia Neutralizer

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Abstract

A study was conducted to test the performance of a high-density (>3000 individuals/mL) continuous recirculating system for rotifers (*B. rotundiformis*) fed nonviable *Nannochloropsis oculata* and using sodium hydroxymethanesulfonate to neutralize ammonia. Three different microalgae feed rates (g of *N. oculata* [68 × 10^9 cells/mL] per million rotifers/d) were tested in successive trials. In Trial 1 (feed rate = 1.5), during a 30-d period, rotifers were harvested daily to 3000 individuals/mL, for an average yield of 178 million/d. Feed efficiency (million rotifers/g/d) was 0.33. In Trial 2 (feed rate = 1.1), during a 32-d period, an average of 106 million rotifers were harvested daily, and feed efficiency was 0.26. In Trial 3 (feed rate = 1.3), during a 30-d period, an average of 107 million rotifers was harvested daily, and feed efficiency was 0.23. An economic analysis based on a feed rate of 1.5 showed that production cost was 40% lower than the traditional batch culture method (US$ 0.29 vs. 0.46 per million rotifers/d). The continuous culture system tested reliably produced large quantities of rotifers on a daily basis without the use of a biofilter and with a lower production cost than a batch culture system.

One of the factors limiting seed production in a marine finfish hatchery operation is the production of the rotifers *Brachionus rotundiformis* and *B. plicatilis* as a food source for first feeding larvae. It has been estimated that 400 billion rotifers are required to produce 10 million gilthead seabream, *Sparus aurata*, fry in Mediterranean hatcheries (Zmora et al. 1991 cited by Lubzens et al. 2001). Many marine finfish hatcheries rely on the traditional "batch culture" method for rotifer production in which several tanks are maintained and harvested at timed intervals to ensure a continual supply. This method is labor intensive, requires a large portion of a hatchery's floor space, and is often unstable and unpredictable (Dhert et al. 2001). High-density continuous rotifer culture systems are currently being developed worldwide to provide daily harvests from one tank. These systems, utilizing microalgae as feed, have been shown to significantly reduce hatchery space requirements and labor, eliminating the need for multiple tanks, and improve culture stability by stabilizing the bacterial populations in the culture (James and Abu-Rezeq 1989; Fu et al. 1997; Yoshimura et al. 1997; Rombaut et al. 2001). Recently, a recirculating system for continuous high-density rotifer culture was described (Suantika et al. 2000, 2003). This system not only reduced water consumption but also replaced microalgae with a cost-effective formulated diet.

For an intensive culture of rotifers to operate efficiently, there must be an adequate supply of food to maintain the rotifers in a satiated condition (Hirayama et al. 1973). The development of commercially available concentrated microalgae has allowed the culturist to provide the rotifers with sufficient amounts of feed without the need to produce live microalgae in the hatchery (Lubzens et al. 1995). This has greatly reduced the need to allocate time and space to live feed production; however, the use of concentrated microalgae in high-density rotifer culture still represents 86% of production costs (Fu et al. 1997).

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Increasing levels of NH_3 within the culture medium, associated with rotifer population growth, have a negative effect on the reproductive rate of both *B. rotundiformis* and *B. plicatilis* (Yu and Hirayama 1986; Araujo et al. 2001). One method for reducing NH_3 levels in a culture is to regulate the pH at 7.0 through the addition of hydrochloric acid using an automated pH controller (Yoshimura et al. 1996). This method keeps the majority of total ammonia nitrogen (TAN) as nontoxic NH_4^+ and has been used to successfully culture rotifers at densities from 10,000 to 30,000 individuals/mL. Alternatively , a specialized micropore membrane filtration device has been used to remove water containing NH_3 from the culture without removing algal cells or rotifers, allowing ultrahigh densities (1.6×10^5 individuals/mL) to be maintained for 4 d in a flow-through culture system (Yoshimura et al. 2003). Although not practical in a commercial operation, this system showed that extremely high densities are possible if ammonia is removed. A preconditioned biological filter has also been used to remove ammonia in intensive rotifer culture to maintain cultures at 3000 individuals/mL for 34 d (Suantika et al. 2000). A new method to reduce ammonia in rotifer culture involves adding sodium hydroxymethanesulfonate to the culture water which neutralizes unionized ammonia and its toxic effect. This product was effective in decreasing TAN and NH_3 in a small-scale rotifer batch culture system (Riche and Pfeiffer 2006). To date, no studies have demonstrated the successful application of sodium hydroxymethanesulfonate in batch culture at a practical scale or in continuous, high-density culture systems.

The unpredictability of rotifer cultures is a significant problem among many hatcheries and has been attributed to degrading water quality and unstable bacterial populations within the culture (Dhert et al. 2001; Rombaut et al. 2001). Continuous culture systems have improved the stability of rotifer cultures (Fu et al. 1997; Suantika et al. 2000; Dhert et al. 2001; Hagiwara et al. 2001; Rombaut et al. 2001). However, in a recent survey of 13 hatcheries in Europe, where rotifer culture ranging from batch culture to recirculating systems are used, human error was suspected to be the most prevalent reason for culture failures (Dierckens 2005). It is important, therefore, to reduce labor associated with rotifer culture systems and to simplify system components to minimize the potential for human error.

The objective of this study was to evaluate the performance of a simplified recirculating system using condensed, nonviable microalgae *Nannochloropsis oculata* and using sodium hydroxymethanesulfonate to neutralize ammonia.

Methods

This study was conducted from February to August 2004 at the University of North Carolina Wilmington Center for Marine Science (UNCW-CMS) Aquaculture Facility, Wrightsville Beach, North Carolina, USA. The UNCW-CMS stock culture of *B. rotundiformis* was used for this study. The same procedures were used for all three trials.

Culture System

The rotifer culture system was situated in an indoor, controlled environment laboratory. The system consisted of a semisquare, rounded corner 190-L culture tank with a sloping bottom and containing 125-L of culture water (Fig. 1).

FIGURE 1. *Intensive system with 50-L sump on left, 150-L culture tank in middle, and feed cooler on right.*

Seawater was pumped from a natural source adjacent to the lab. Culture water was prepared by mixing 1-μm filtered, UV-sterilized seawater with municipal freshwater to achieve a salinity of 20 ppt. For further sterilization, the culture medium was chlorinated for 24 h with liquid bleach solution (6.5% ClONa) and then dechlorinated with sodium thiosulfate.

The culture tank was fitted with a center standpipe drain consisting of a 10-cm-diameter plastic netting frame covered with a 55-μm nylon mesh screen to retain the rotifers in the culture vessel and an inner standpipe to maintain the culture volume (Fig. 2). A bubble ring at the bottom of the standpipe decreased fouling of the mesh. Twenty-six liters per hour (500%) of system water was recirculated through the culture tank each day. Water exiting the culture tank drained by gravity into a 50-L sump tank, and water was then circulated by a 1.5-amp pump through a foam fractionator at a rate of 24.2 L/min. to remove dissolved and suspended solids. A bypass diverted the remainder of the flow into the sump. The total system volume was 220 L (Fig. 3).

Aeration was provided to the culture tank from a blower through four 4-cm air diffusers, and pure oxygen was delivered from a dewar through a 15-cm microdiffuser. Temperature was controlled by a 300-W glass submersible heater placed in the culture tank and was maintained at 25.0–29.9 C. Natural lighting was provided through a laboratory window, and fluorescent overhead lights were used only during system maintenance.

Feeding

To begin a rotifer culture trial, the system was initially stocked at a density of 600–900 individuals/mL and the culture density was monitored daily. Nonviable microalgal paste *N. oculata* (approximately 68×10^9 cells/mL) (Reed Mariculture, Campbell, CA, USA) was used to feed the rotifer culture. Approximately 10 g was added directly to the culture tank, and the remainder of the algae paste was diluted in 16 L of 20 ppt water and stored in a cooler with ice packs. The diluted algal paste was added continuously to the culture tank over a 22-h

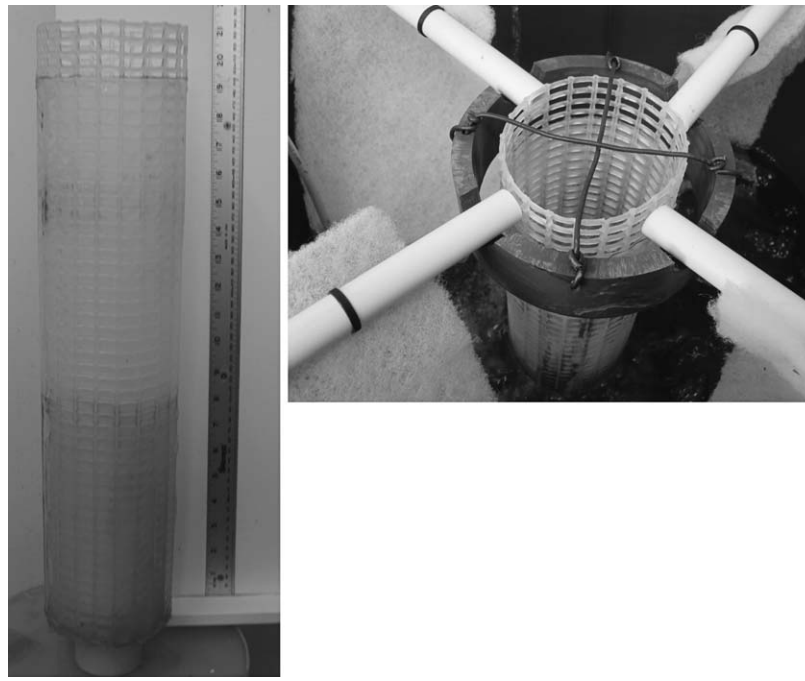

FIGURE 2. *Center drain for culture unit with 55-μm screen. Four filter mats are suspended from center drain.*

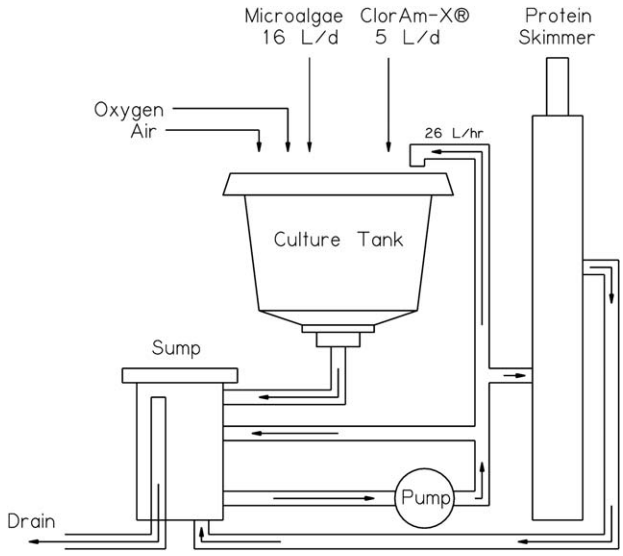

FIGURE 3. *System schematic depicting pipe configuration and water flow.*

period by a peristaltic pump at a flow rate of 12 mL/min.

Harvesting

Once the rotifer population reached >3000 individuals/mL, the culture was harvested daily to reduce the population to 3000 individuals/mL. Rotifers were harvested by draining the appropriate volume of water to reduce the rotifer density to 3000 individuals/mL at full volume. If the culture density fell below 3000 individuals/mL, a minimum of 2% of the culture was harvested. The culture was maintained for 39 d, which was considered to be a sufficient time period to demonstrate long-term stability of the culture system.

Water Conditioning

To neutralize toxic-free ammonia in the culture water, a commercially available water conditioner, sodium hydroxymethanesulfonate (ClorAm-X®, AquaScience Research Group, Inc., North Kansas City, MO, USA) (Kuhns 1987) was added daily at a rate of 0.25 g/10^6 rotifers. The ClorAm-X was dissolved in 5 L of 20 ppt water and dripped into the water from a container suspended over the tank over a 24-h period. A small valve was used to control the drip rate into the tank. ClorAm-X is an alkali metal formaldehydebisulfite that binds to unionized ammonia creating an aminomethanesulfonate salt and thereby reducing TAN. This molecule is nontoxic and does not interfere with the nitrogen cycle (Kuhns 1987). The neutralizing of free ammonia releases a hydrogen ion, lowering pH. To buffer the system, 5 g of sodium bicarbonate/d was added to the ClorAm-X solution.

Water Quality

To assist in the removal of suspended solids from the culture tank, four 8-mm viline mats (0.25 × 0.64 m) were suspended in the water column and were removed and rinsed daily. In addition, a small airlift pump was placed in the middle of the tank, which circulated culture water through a viline canister filter. Approximately 2–3 L of the culture was siphoned from the bottom daily to remove settled solids. To conserve rotifers, the siphoned water was filtered through a piece of vilene mat, and the filtrate was then returned to the tank.

Daily water exchange comprised water required to replace harvest volume (5–55 L) and water added along with diluted *N. oculata* (16 L) and ClorAm-X (5 L). As a precautionary measure, the entire population was removed from the system every 14–23 d, allowing the system to be cleaned, filled with new water, and then restocked at 3000 individuals/mL.

Temperature, dissolved oxygen (DO), salinity, and pH were measured daily. The ammonia was measured periodically by filtering the sample through a 0.45-μm filter to remove all photoreactive substances and tested with a Hach Nitrogen, Ammonia, High Range, Test′N Tube salicylate test kit (Hach Company, Loveland, CO, USA, Method 10031) adapted for a Spectronic 20D+. NH_3 was calculated according to Emerson et al. (1975).

Experimental Design

To optimize feed costs, three different microalgae feed rates were examined in successive trials. In the first trial, rotifers were fed 1.5 g of *N. oculata*/million rotifers/d. In the second trial, rotifers were fed 1.1 g of *N. oculata*/million rotifers/d. A third trial tested an intermediate feed rate of 1.3 g of *N. oculata*/million rotifers/d. Treatment feeding rates were initiated once the culture reached a density of 3000 individuals/mL. During the startup period, slightly higher feed rates were used to ensure adequate feeding of the rotifer culture.

Sampling

To monitor rotifer population growth and culture density, the rotifer culture was sampled daily by removing ten 1-mL samples from different areas of the tank and diluting the combined samples to 100 mL with seawater. Rotifers were counted in three separate l-mL samples using a multiwell depression slide with a stereo microscope. Lugol solution was added before counting to immobilize and stain the rotifers. Rotifers that appeared clear were considered dead and were not counted. Average rotifer density of the three 1-mL subsamples were calculated, multiplied by 10 to account for sample dilution, and multiplied by the tank volume (120 L) to estimate total population.

Data Analysis

To determine the efficiency of the feed rates, the amount of algae required to produce 1×10^6 rotifers was calculated for each harvest day, and an overall mean was calculated for the duration of the study.

Results

Trial 1

During the first trial, a feeding rate of 1.5 g of *N. oculata* paste/million rotifers/d was used, and the culture system was stocked at a density of 633 individuals/mL (population = 76.0×10^6). The density reached 5290 individuals/mL (population = 634.8×10^6) by Day 10, and 6500 individuals/mL (population = 780×10^6) by Day 12 (Fig. 4). Beginning on Day 12, the culture was harvested daily to maintain a baseline density of 3000 individuals/mL at full volume. On Day 23, the system was cleaned, filled with new water, and restocked at 3000 individuals/mL. On Day 31, the density fell to 2970 individuals/mL (population = 356.4×10^6); however, 8% (30×10^6 rotifers) of the culture was harvested, lowering density to 2717 individuals/mL (population = 326×10^6). On Day 35, culture density was 3150 individuals/mL, but 25% (94.5×10^6 rotifers) was harvested, lowering density to 2363 individuals/mL. In Trial 1, mean ± SD daily yield over the 30-d harvest period was $178 \times 10^6 \pm 81.1 \times 10^6$ rotifers and ranged from a minimum of 30×10^6 (Day 31) to a maximum of 360×10^6 (Day 12) (Fig. 4). Feed efficiency (million rotifers/g/d) was 0.33 ± 0.15.

During Trial 1, mean TAN concentration was 25.6 ± 5.8 mg/L, and the mean NH_3 concentration was 0.09 ± 0.07 mg/L. The pH during Trial 1 averaged 6.6 and ranged from 6.2 to 7.3 (Table 1). Mean DO levels for Trial 1 was 10.4 ± 3.7 mg/L, while mean salinity for Trial 1 was 19.7 ± 1.6 ppt (Table 1).

Trial 2

In a second trial, using a feed rate of 1.1 g of *N. oculata* paste/million rotifers/d, initial stocking density was 841 individuals/mL (population = 100.9×10^6), with density reaching 4340 individuals/mL (population = 520.8×10^6) by Day 8 when daily harvesting to 3000 individuals/mL began (Fig. 4). On Day 9 and Day 23, the culture was harvested to 2500 individuals/mL and 2650 individuals/mL, respectively. In Trial 2, mean daily harvest over the 32-d harvest period was $106 \times 10^6 \pm 57.6 \times 10^6$ rotifers,

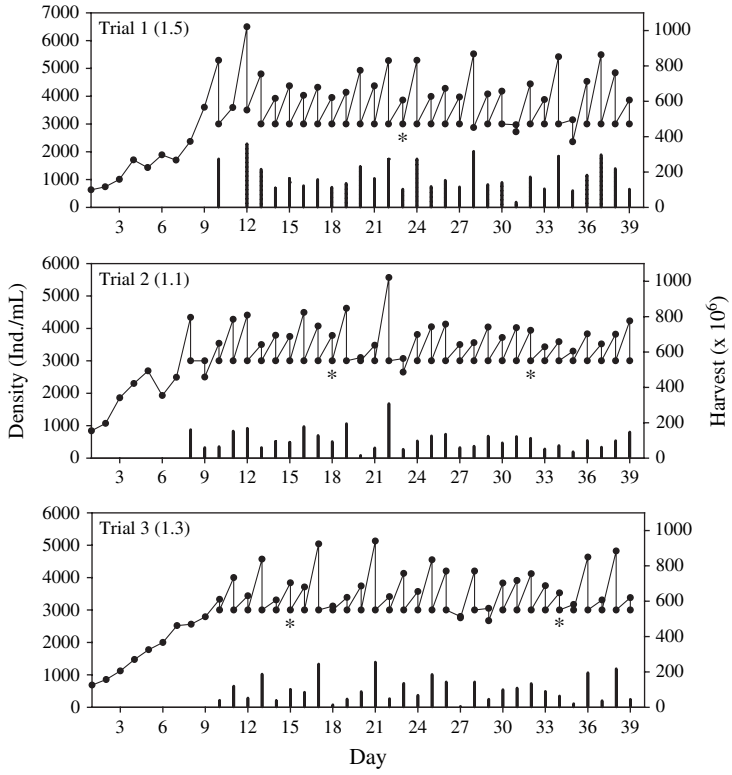

FIGURE 4. *Culture densities and daily rotifer production for the three feed rate trials. Bars represent number of individuals harvested and asterisks represent system cleaning and restocking.*

ranging from a minimum of 16×10^6 rotifers (Day 20) to a maximum of 308×10^6 rotifers (Day 22) (Fig. 4). Feed efficiency was 0.26 ± 0.14. On Day 18 and Day 32, the system was cleaned, filled with new water, and then restocked at 3000 individuals/mL.

During Trial 2, mean TAN concentration was 24.9 ± 6.0 mg/L, and mean NH_3 concentration was 0.10 ± 0.09 mg/L (Table 1). The mean pH, DO, and salinity during Trial 2 were 6.6 (range = 5.3–8.0), 9.2 ± 3.1 mg/L, and 19.7 ± 1.7 ppt, respectively (Table 1).

Trial 3

In a third trial, using a feed rate of 1.3 g of *N. oculata* paste/million rotifers/d, initial stocking density was 685 individuals/mL (population = 82.2×10^6), with density reaching 3330 individuals/mL (population = 399.6×10^6) by Day 10 (Fig. 4). Daily harvests to 3000 individuals/mL began on Day 10. On Day 27 and Day 29, the culture was harvested to 2756 individuals/mL (population = 330.7×10^6) and 2669 individuals/mL (population = 320.2×10^6),

TABLE 1. *Water quality parameters for three trials conducted in a continuous culture rotifer system.*[a]

Parameter	Trial 1	Trial 2	Trial 3
Temperature (C)	28.2 ± 0.9 (25.2–29.4)	28.0 ± 0.7 (25.0–29.0)	28.8 ± 0.9 (26.4–29.9)
TAN (mg/L)	25.6 ± 5.8 (16.5–37.9)	24.9 ± 6.0 (17.5–32.2)	35.4 ± 8.4 (22.8–48.0)
NH_3 (mg/L)	0.09 ± 0.07 (0.02–0.21)	0.10 ± 0.09 (0.01–0.11)	0.06 ± 0.04 (0.01–0.28)
pH	6.6 (6.2–7.3)	6.6 (5.3–8.0)	6.6 (5.9–7.7)
Dissolved oxygen (mg/L)	10.4 ± 3.7 (3.3–16.7)	9.2 ± 3.1 (4.0–16.6)	9.5 ± 4.8 (3.5–19.6)
Salinity (ppt)	19.7 ± 1.6 (16.0–22.0)	19.7 ± 1.7 (17.0–24.0)	17.8 ± 1.8 (14.0–21.0)

[a] Values represent mean ± standard deviation (range).

respectively. In Trial 3, mean daily harvest during the 30-d harvest period was $106.7 \times 10^6 \pm 72.2 \times 10^6$, ranging from a minimum of 5×10^6 rotifers (Day 27) to 256×10^6 rotifers (Day 21) (Fig. 4). Feed efficiency was 0.23 ± 0.15. The system was cleaned, filled with new water, and restocked on Day 15 and Day 23.

In Trial 3, mean TAN concentration was 35.4 ± 8.4 mg/L, and mean NH_3 concentration was 0.06 ± 0.04 mg/L (Table 1). Mean pH during Trial 3 was 6.6 (range = 5.9–7.7). Mean DO was 9.5 ± 4.8 mg/L, while salinity was 17.8 ± 1.8 ppt (Table 1).

Discussion

Benefits of System

In this study, a continuous culture of *B. rotundiformis* was maintained by using a recirculating system and feeding, nonviable, condensed microalgae. Culture densities of 3000–6500 individuals/mL were maintained, with daily harvests averaging 1.48×10^9 individuals/d/m^3 for more than 30 d. On Days 31 and 35 in Trial 1, Day 23 in Trial 2, and Day 27 and Day 29 in Trial 3, densities fell below 3000 individuals/mL. These declines in density were not associated with any of the monitored water quality parameters and the causes were not determined. Fu et al. (1997) demonstrated the benefits of a continuous culture system for *B. rotundiformis* using the condensed microalgae *Chlorella vulgaris* maintaining culture densities ranging from 3000 to 6000 individuals/mL, with an average daily production of 2.1×10^9 individuals/d/m^3 over 110 d. This system significantly reduced labor and hatchery space required for live food production but required a water exchange rate of 60–70% of the culture volume per day to maintain good water quality conditions (Fu et al. 1997). Suantika et al. (2003) maintained culture densities ranging from 3000 to 6500 individuals/mL, with an average daily harvest of 2.2×10^9 individuals/d/m^3 over 27 d. This was accomplished in a recirculating system using a formulated rotifer feed, which necessitated the use of ozone, multiple foam fractionators, and a preconditioned biofilter to maintain water quality (Suantika et al. 2003). In the present study, an average makeup water exchange rate of 28% of the culture volume per day (60.6 L/d) was used (Table 2), and recirculation system components (including a biofilter and ozone generator) were eliminated through the use of nonviable microalgae and a commercial water conditioner (ClorAm-X) to neutralize ammonia. In addition, a foam fractionator was used for removal of dissolved solids and fine particulate matter. The rotifers produced in this system were of high quality and were successfully used for the production of marine finfish larvae (Mangino and Watanabe 2006).

Sodium Hydroxymethanesulfonate

For successful rotifer production, it is essential to reduce the concentration of unionized ammonia in the culture medium because elevated levels significantly decrease both the fecundity and the lifespan of rotifers (Araujo et al. 2001). One approach to reducing the concentration of unionized ammonia in rotifer recirculating systems is to use a biofilter or a biofilter in combination with ozone (Suantika et al. 2000, 2001, 2003). This method worked well and was capable of maintaining low ammonia levels (<0.8 mg/L NH_4^+) with the use of commercially formulated feed (Suantika et al. 2003). However, a 6-d biofilter conditioning period was required prior to inoculating the system with rotifers (Suantika et al. 2001). The need to precondition a biofilter may complicate the start up and cleaning of the system, and it may be difficult to adjust culture densities to match the rotifer production needs of a hatchery, which may vary considerably from week to week. The additional equipment also adds complexity to the system and increases the system cost.

Rotifers have been successfully cultured at high densities ($22–34 \times 10^3$ individuals/mL) in the presence of TAN levels as high as 1000 mg/mL by maintaining a pH of 7 through the automated addition of HCl and NaOH (Yoshimura et al. 1996). In this study, ClorAm-X was added continuously to the rotifer culture to neutralize toxic-free ammonia, thereby reducing the amount of TAN and to maintain a lower pH (mean pH of 6.6 for all three trials), allowing a healthy culture

TABLE 2. *Comparison of daily inputs and rotifer production costs in batch and intensive culture systems.*[a]

	Batch system	% Cost	Intensive system	% Cost
Inputs				
Feed rate (g/million)	0.75		1.50	
N. oculata (g)	164.3		482.0	
ClorAm-X[b] (g)	54.9		81.9	
Labor (hr)	3.0		1.0	
Electricity (kWh)	7.20		7.84	
Water usage (L)	125.0		60.6	
Oxygen (ft^3)	na		19.2	
Investment cost (US$)	830.00		995.00	
Annual depreciation[c] (US$/yr)	129.60		185.50	
Cost (US$)/$10^6$ rotifers				
N. oculata[d]	0.09	20.45	0.17	58.1
ClorAm-X[e]	0.01	1.5	0.01	2.1
Labor cost[f]	0.29	62.35	0.06	20.1
Electricity[g]	0.0039	0.9	0.0026	0.9
Oxygen[h]	na	na	0.0323	11.0
Water[i]	0.0636	13.9	0.0199	6.8
Depreciation cost	0.0034	0.7	0.0030	1.0
Total cost per million	0.457		0.293	

na = not applicable.

[a] Costs were per million rotifer/d, based on an average daily production of 170×10^6 rotifers/d for the intensive system and on an average daily production of 106×10^6 rotifers/d from four batch tanks using a 4-d harvest cycle.

[b] Sodium hydroxymethanesulfonate.

[c] Calculated from Table 3.

[d] US$ 0.06/g.

[e] US$ 0.013/g.

[f] US$ 10.00/h.

[g] US$ 0.06/kWh.

[h] Includes cost for bottle rental (US$ 15.00/d) and gas cost (US$ 0.26/ft^3).

[i] Includes artificial sea salt, municipal water, sodium hypochlorite, sodium thiosulfate (US$ 0.052/L), and depreciation of pump and two reservoirs for mixing and holding water (US$ 0.224/d).

to be maintained in the presence of high TAN levels. Mean NH_3 levels during all three feed rate trials (0.06– 0.10 mg/L) were well below the levels that were determined to negatively affect population growth (2.1 mg/L) and reproductive rate (2.4 mg/L) for *B. plicatilis* (Yu and Hirayama 1986). The use of ClorAm-X in this system permitted continuous daily harvests without the use of a biofilter and its associated problems and eliminated the reliance on an automated pH adjustment system. This allows the culturist more flexibility to rapidly increase or decrease the culture density according to demand and may decrease the potential for culture declines because of equipment failure.

The system was cleaned and restocked every 14–23 d to prevent the build up of detritus. While this may not have been necessary, because the system continued to produce a normal harvest on the day after cleaning and restocking, this precautionary measure was simple and considered worthwhile.

Nonviable Condensed Microalgae

The use of nonviable condensed *N. oculata* for intensive rotifer culture has a number of advantages. It is commercially available and it can be stored long term (>4 wk) at −20 C without detrimental affects on nutritional quality (Lubzens et al. 1995). It also eliminates the labor and large amount of hatchery space required for maintaining live algal cultures to feed rotifers. However, when production is scaled up to meet the requirements of a commercial hatchery, the cost of nonviable microalgae may be prohibitive. Fu et al. (1997) showed an increase in feed cost in a continuous culture system over traditional batch culture using a commercially available

condensed microalgae. However, Suantika et al. (2003) reported that use of a commercially formulated artificial diet in a recirculating system for rotifers reduced feed costs by 21% compared to traditional batch culture methods. The use of a formulated microdiet in conjunction with an ammonia neutralizer merits evaluation.

Microalgae Feed Rate Trials

In this study, feeding rates of 1.1, 1.3, and 1.5 g of *N. oculata* paste/million rotifers/d in three separate 39-d trials yielded from 106 to 178 million rotifers/d. The different feeding rates were studied in consecutive trials, precluding statistical comparisons among treatments. However, at the highest mean daily rotifer production rate (178 million/d) *N. oculata* was added at a rate of 102×10^9 cells/million rotifers/d. This is higher than feed rates reported in other high-density continuous rotifer culture systems using condensed microalgae. Yoshimura et al. (1997) fed *C. vulgaris* at a rate of 75×10^9 cells/million rotifers/d, and Fu et al. (1997) fed *C. vulgaris* at a rate of approximately 72×10^9 cells/million rotifers/d. The lower feed rates reported by these authors may be because of the larger cell size (2–10 μm) of *C. vulgaris* compared to *N. oculata* (2–4 μm) (Maruyama et al. 1989; Fott and Novakova 1969 cited by Maruyama et al. 1997).

Economics

For economic analysis, Trial 1 (feed rate = 1.5) was used to calculate overall production cost and then compared with production costs associated with a batch culture system. For these analyses, it is assumed that artificial seawater is used. For the batch culture system, production costs were based on mean yield for three batch culture cycles (unpublished data, Bentley, UNCW). The batch culture system consisted of a 190-L tank and followed a 4-d culture cycle. The culture cycle began on Day 0 at a density of 400 individuals/mL in 50 L of 21 ppt water, with 25 L of water added per day until the culture was harvested on Day 4. Rotifer production was determined after removing 20×10^6 rotifers from the harvest for inoculation of the next cycle. For the continuous culture system (this study), the overall production cost (feed, labor, electricity, oxygen, water, and depreciation) per million rotifer/d was US$ 0.29 (Table 2), significantly higher than calculated for other intensive rotifer culture systems, which ranged from US$ 0.03–0.04 per million rotifers/d (Fu et al. 1997; Suantika et al. 2003). Production cost per million rotifer ($0.29) was 40% lower than batch culture methods ($0.46) (Fig. 5; Table 2). The greatest reduction in cost was because of a 79% reduction in labor. This is consistent with the intensive system developed by Suantika

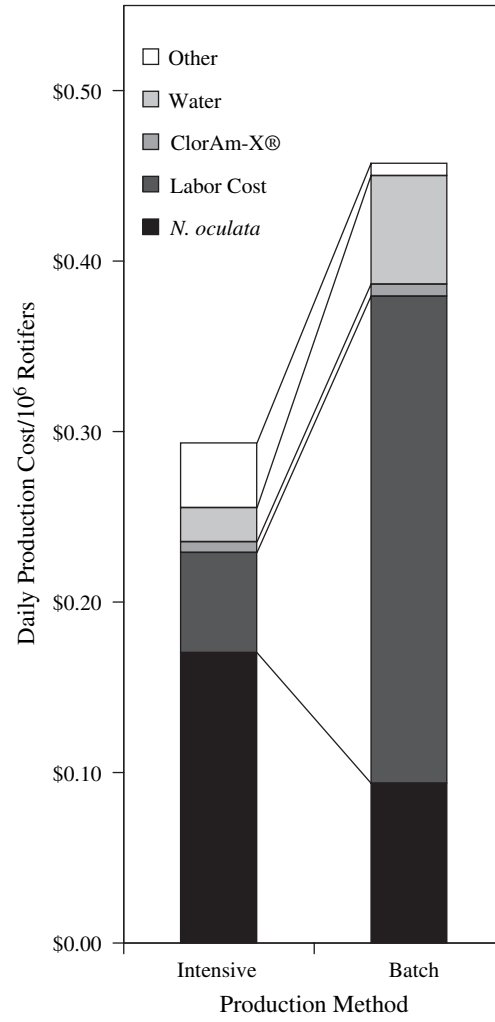

FIGURE 5. *Comparison of daily production cost per million rotifers in an intensive culture system and in a batch culture system. See Tables 1 and 2 for details of each system.*

TABLE 3. *Total system cost and annual depreciation of equipment for an intensive system and a batch system.*

Item	Quantity	Unit cost (US$)	Total cost (US$)	Useful life (yr)	Annual depreciation (US$)
Intensive system					
Tank (190 L)	1	84.00	84.00	10	8.40
Stand	1	112.00	112.00	10	11.20
Sump (50 L)	1	55.00	55.00	10	5.50
Protein skimmer	1	250.00	250.00	10	25.00
Pump	1	295.00	295.00	5	59.00
Heater (300 W)	1	12.00	12.00	1	12.00
Feed pump	1	100.00	100.00	2	50.00
Feed cooler	1	30.00	30.00	10	3.00
Oxygen diffuser	1	52.00	52.00	5	10.40
Air diffuser	5	1.00	5.00	5	1.00
Total			995.00		185.50
Batch system					
Tank (190 L)	4	76.50	306.00	10	30.60
Stand	4	112.00	448.00	10	44.80
Heater (300 W)	4	12.00	48.00	1	48.00
Oxygen diffuser	na	na	na	na	na
Air diffuser	4	7.00	28.00	5	5.60
Total			830.00		129.00

na = not applicable.

et al. (2003), which reduced labor by 65% compared to batch culture. In this study, there was a significant increase (45%), however, in the feed cost for the intensive system attributable to feed lost via the foam fractionator. The hatchery floor space required for the intensive rotifer culture system was also significantly reduced (34%) when compared to batch culture. There was a slight increase in depreciation of the intensive system over batch culture (Table 3). The elevated feed cost in this system when compared to other continuous systems may be a result of differences in the availability of feed among studies.

The system presented here reliably produced large quantities of rotifers on a daily basis without the use of a biofilter. This allowed greater flexibility by eliminating the need for biofilter conditioning. The reduction in operational cost provided significant advantages over traditional batch culture methods in a commercial situation. The reliability of the culture was also greatly improved by eliminating the frequent handling of the culture required for batch culture methods, which may add stress and increase the chance of a failure because of human error. Further reductions in operational costs may be attained by examining alternative feeds.

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