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High-density production of the rotifer *Brachionus plicatilis* in a recirculation system: consideration of water quality, zootechnical and nutritional aspects

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Abstract

Rotifers were reared on the artificial diet culture Selco® in batch and recirculation conditions at different water exchange rates. The different rearing conditions resulted in considerable changes in water quality, which in their turn affected rotifer growth and food consumption. At a daily water exchange rate of 100%, no positive effect was obtained in rotifer growth compared to the batch rearing system, but the rotifer culture period could be prolonged by 1 week. By increasing the daily water exchange rate from 100 to 300% the maximum rotifer density could be significantly ($P < 0.05$) increased from 1800 to 2500 individuals ml^{-1}. At the highest recirculation rate (daily water exchange of 500%) the highest rotifer production (2800 individuals per milliter in 11 days) was obtained after adjustment of the feeding scheme. This adjustment was necessary to compensate for food losses in the recirculation system. The use of a modified culture Selco (CSH) could further improve the performance of the rotifers. Using this experimental diet, a rotifer density of 8000 individuals per milliter could be obtained in 8 days without rinsing and restocking during the production period. When the rotifer populations were kept below their maximal density by daily harvests the culture period could be extended to more than 1 month. During this period the cultures were not subjected to any water exchange or restocking except the replacement of the water to compensate for the daily harvested rotifers ($\pm 20\%$ of the standing population). In general terms it can be stated that the use of a recirculation system has proved to reduce labour and maintenance cost while ensuring stable physico-chemical rearing conditions resulting in more reliable and healthy rotifer cultures.

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1. Introduction

The successful development of commercial farms in the Mediterranean area has been made possible by several improvements in the production techniques of the live food (Sorgeloos and Sweetman, 1993; Candreva et al., 1996; Dehasque et al., 1997). This success is not only attributable to *Artemia* but also to the brackish water zooplankton *Brachionus plicatilis*, which is universally used as starter diet (Fukusho, 1989).

The expansion of the size of commercial hatcheries has increased their daily requirements for rotifers to such an extent that it becomes a burden for the further industrialisation of the larviculture process. Moreover, the unpredictability in rotifer mass production forces hatcheries to keep several simultaneous cultures and back up cultures to provide enough food for feeding their fish larvae (Candreva et al., 1996). In most European hatcheries rotifers are generally reared in batch systems and fed algae or a mixture of algae and baker's yeast or on culture Selco®. Under these batch culture conditions rotifers are subjected to repeated water renewals to maintain an acceptable water quality. It is not surprising that under these stressful rearing conditions the rotifer and the rotifer density in commercial systems is seldom exceeding 600 individuals per milliter (Fukusho, 1983; Morizane, 1991). Recently, new culture methods in rotifer production have been developed enabling high density populations in continuous cultures (Abu-Rezq et al., 1997; Fu et al., 1997; Yoshimura et al., 1997) but all of these culture methods rely on the use of concentrated suspensions of marine algal species.

The challenge of this study consisted in designing a high density rearing technique using an artificial diet instead of condensed or concentrated marine algae for the rotifer *B. plicatilis*. For this an appropriate recirculation system was designed to maintain an optimal water quality during the rearing procedure and hence reduce manipulation and stress of the rotifer population.

2. Materials and methods

2.1. Rotifer strain

All experiments were performed with *B. plicatilis* (L-strain with lorica length, 180 ± 15 μm). Before the start of the experiment, the rotifer strain was kept in culture for 6 months at the Laboratory of Aquaculture and Artemia Reference Center, following the culture procedure described in Sorgeloos and Lavens (1996).

2.2. Experimental set up

In all experiments the rotifers were inoculated at a density of 250 individuals per milliter in 100-l cylindro-conical tanks in three replicates per treatment. The culture water consisted of diluted seawater (25 ppt salinity), temperature was maintained constant at 25 ± 1°C.

A schematic outline of the batch culture system is presented in Fig. 1. The batch culture system was performed following the culture procedure described in Sorgeloos and Lavens (1996). In this batch culture system rotifers were stocked at a starting density of 250 individuals per milliter and rinsing and restocking was performed every 4 days. Fig. 2 gives a schematic outline of the recirculation culture system (RC) that was used. The rotifers were maintained in identical rearing tanks as for the batch culture but the tanks were equipped with a central nylon screen with a mesh size of 33 μm. The airstones were replaced by an aeration collar enclosed on the outer bottom part of the filter. In order to ensure a continuous cleaning of the filter the aeration was maintained at 2.1 ± 0.2 l min^{-1}. This aeration also provided a homogenous mixing of the rotifers and their food. The effluent water from the three replicated cultures was collected in a 100-l settlement tank before being treated in a protein skimmer. This protein skimmer had a capacity of 55 l and a maximum water flow rate of 1200 l h^{-1} (DBPr. 35 25 861 AquarienTechnik Klaes, Germany). Suspended organic matter (e.g. excess food, flocules, ciliates) from the effluent water was trapped in the foam of the protein skimmer and

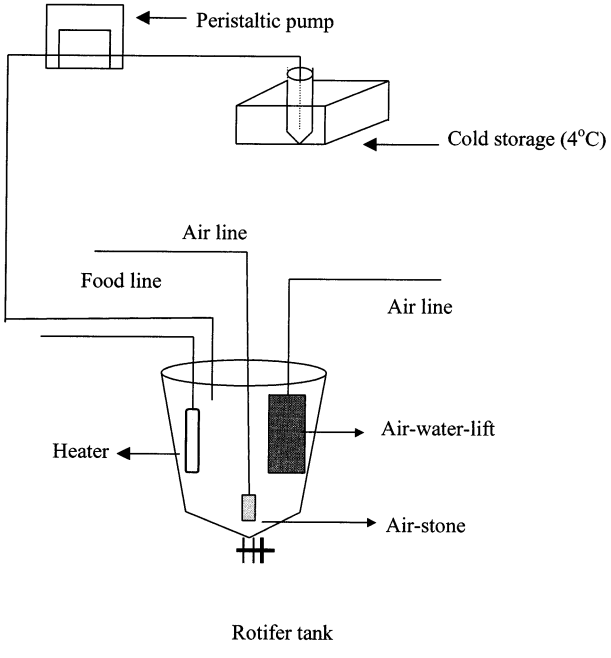

Fig. 1. Schematic overview of the batch culture system.

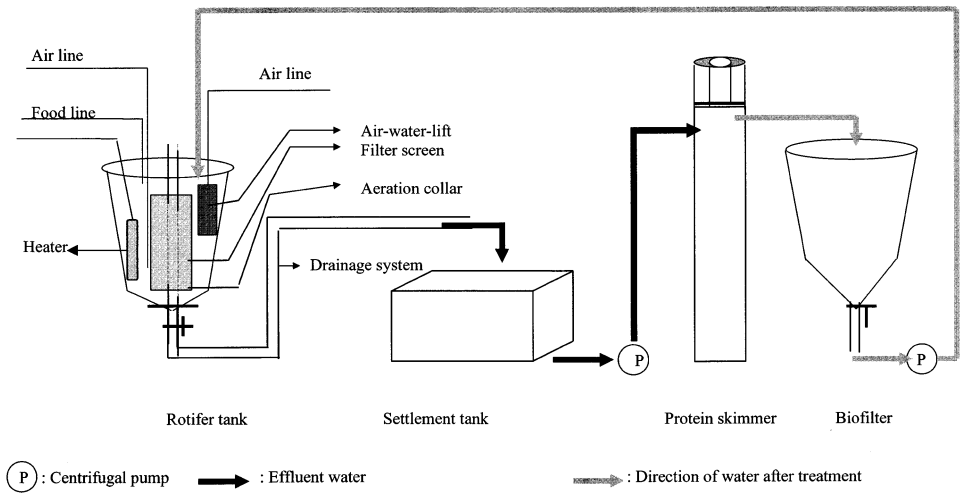

Fig. 2. Schematic overview of the recirculation system.

Table 1
Composition of the nitrification medium used for the culture of nitrifying bacteria

	($g\ l^{-1}$)
NH_4Cl	Variable (0.5–1.0 N–NH_4Cl)
$CaCO_3$	0.4
KH_2PO_4	0.16
Trace element solution	0.08
Synthetic seawater (instant ocean®)	25

removed daily. After the physical separation in the protein skimmer, the effluent water underwent a biological filtration in a submerged biofilter with a capacity of 90 l filled with 60 l gravel substrate (size = 3–8 mm). The biofilter was inoculated with an enriched culture of nitrifying bacteria. During several months, these autotrophic bacteria were cultured in a continuously aerated and pH-controlled bioreactor, fed on a nitrification-feeding medium (Table 1). After the biological filtration the treated water was reinjected in the rotifer cultures. The effect of different water exchange rates on the growth of the rotifer population was investigated by increasing the water renewal in the culture tanks from 100 (i.e. 100 $l\ day^{-1}$) to 300 and 500%. The flow rate on the biofilter also depended on the water renewal rate of the culture water and was two times higher than the water renewal rate in the tanks. The optimisation of the feeding scheme using culture Selco® (CS) and an experimental diet CSH was performed in the recirculation system with a daily water exchange rate of 500% starting from 500 individuals per milliter. Experiments were performed in duplicate or triplicate depending on the availability of tanks. All experiments were conducted until the exponential growth of the rotifer

stopped and which corresponded to the maximum carrying capacity of the recirculation system.

Since the recirculation systems are especially suited for long term rearing an experiment was conducted in which the rotifers were maintained at 3000 individuals per milliter by daily harvests. This experiment was conducted with a recirculation rate of 500% under optimal feeding conditions (see further).

2.3. Sampling and counting

The rotifer densities were counted daily when the food storage tanks were emptied. Three sub-samples of 400 μl were taken from all tanks using an automatic micropipette. Two drops of Lugol were added to the samples to kill the rotifers before counting. Empty and transparent lorica belonging to dead rotifers were not counted. The specific growth rate (SGR) was calculated using the following Eq. (1):

$$\mu = (\ln N_t - \ln N_0)/t \quad (1)$$

where: μ is specific growth rate, N_t is Rotifer density after culture period t (individuals per milliter), N_0 is initial rotifer density (individuals per milliter), t is culture period (day).

2.4. Rotifer diet

In all experiments the commercial rotifer food culture Selco® (CS) was used. This dry food was suspended in 800 ml water and mixed vigorously with a kitchen blender. The suspension containing exactly the daily food ratio was kept in cold storage (4°C) for 24 h. From the cold storage tanks the food was administered automatically by means of a peristaltic pump to the individual rotifer cultures. A standard feeding regime was derived from Lavens et al. (1994) and reduced to the following Eq. (2):

$$\mathrm{CS} = 0.0168 D^{0.415} V \quad (2)$$

where CS is culture Selco® (g), D is rotifer density (individuals per milliter), V is culture water volume (l).

Feeding was performed every hour by means of a peristaltic pump. A timer connected to the peristaltic pump regulated the food distribution at a rate of 33 ml h^{-1} or 800 ml day^{-1} food suspension.

A modified CS formulation (Table 2) with a different particle distribution, higher protein content, higher water stability and lower fat content was also tested in the recirculation system. In a preliminary experiment a tentative feeding scheme was established (results not shown). It appeared that due to the different characteristics of the experimental diet CSH the feeding scheme needed to be modified to Eq. (3):

$$\mathrm{CSH} = 0.027 D^{0.415} V \quad (3)$$

where CSH is experimental diet CSH (g), D is rotifer density (individuals per milliter), V is culture water volume (l).

The optimisation of the standard feeding regimes for both diets at increased water recirculation rates (increased feed losses in the recirculation system) was obtained by correction factors α and β in Eqs. (2) and (3).

2.5. Physico-chemical parameters

pH, NH_4^+, dissolved oxygen and the viscosity of the water were measured as first daily activities during the experiment. NH_4^+ and viscosity measurements were performed on culture water of the rotifer tank filtered through a 30-μm filter. The NH_4^+ concentration was determined using a test kit (Aquamerck®, Germany). The viscosity of the water was measured by the flow time (t) of a 3-ml water sample in a capilar tube (Oswald Viscometer). The kinematic viscosity (v) was calculated by the following Eq. (4):

$$v = K(t - \vartheta) \tag{4}$$

where v is kinematic viscosity, $K = 0.01$, t is flow time (s), $\vartheta = 0.12/Kt$.

The chemical oxygen demand (COD) was measured in the rotifer culture tank, after the protein skimmer and in the biofilter.

2.6. Statistical analysis

All data were checked for homogeneity and normal distribution before being subjected to one-way ANOVA. Significant differences among means ($P < 0.05$) were tested by Duncan's multiple range test.

Table 2
Biochemical composition of the diet culture Selco® and the experimental diet CSH[a]

Composition	Diet	
	Culture Selco® (%)	CSH (%)
Moisture	Max 5	Max 5
Fat	Min 15	Min 9
Protein	Min 35	Min 40
Ash	Max 15	Max 10
Vitamin A	500 IU g^{-1}	500 IU g^{-1}
Vitamin D3	50 IU g^{-1}	50 IU g^{-1}
Vitamin E	3600 mg kg^{-1}	3600 mg kg^{-1}
Particles	1.8×10^{10} cells g^{-1}	2.4×10^{10} cells g^{-1}

[a] Source, INVE, N.V., Belgium.

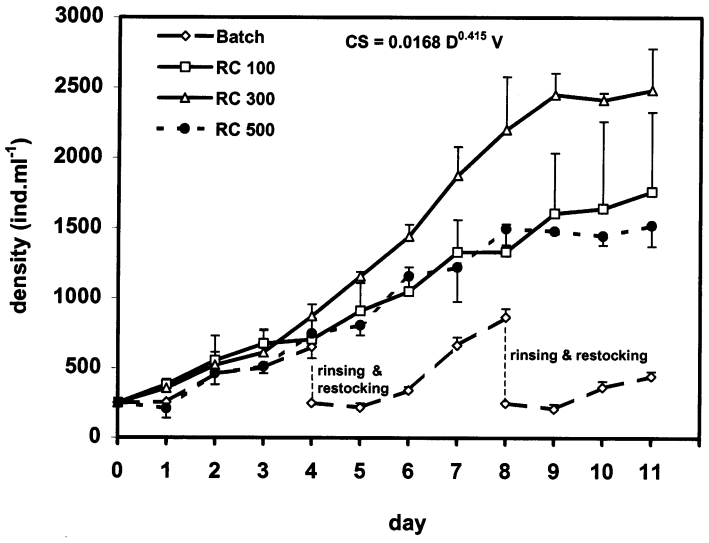

Fig. 3. Rotifer density obtained in the batch and recirculation system with a standard feeding scheme: $CS = 0.0168D^{0.415}V$.

3. Results

3.1. Rotifer growth performance

The average growth of the rotifer populations reared in the batch culture and the recirculation system with the various flow rates [100% recycling per day (RC 100), 300% recycling per day (RC 300), 500% recycling per day (RC 500)] is presented in Fig. 3. In the batch culture, water was renewed once the population growth declined (i.e. after 4 days of culture). After this, the rotifers were rinsed and returned to the system in complete new seawater at a density of 250 individuals per milliter. In the batch system maximum rotifer densities of 860 individuals per milliter were reached at the end of each culture period of 4 days.

In the recirculation system the rotifer cultures were also started at a density of 250 individuals per milliter but no rinsing or water replacement took place. When the rotifer production of the two systems is compared it is clear that in the batch culture system the production is lower especially during the first day after each restocking. For an 11-day culture period this resulted in a total production of 1800 rotifers per milliter in the batch system (i.e. summed production of the three batches: day 0–4, day 4–8, day 8–11). In the recirculation system with low water exchange (RC 100) an identical production was achieved in 11 days. A significant increase in the rotifer density ($P < 0.05$) was obtained by increasing the daily water exchange rate from 100 to 300% resulting in a production of 2500 individuals per milliter at the end of the 11-day culture period. However, increasing the daily water exchange rate in the recirculation system from 300% (RC 300) to 500% (RC 500)

did not result in an increase of the specific growth rate or rotifer density under our standard feeding scheme (Table 3).

Since a lower rotifer production was noticed at increased flow rates under standard feeding conditions a new set of experiments was started in which the recirculation was kept at 500% but where the food distribution was increased. For this, the standard feeding $CS = 0.0168D^{0.415}V$ was modified to $CS = \alpha 0.0168 D^{0.415}V$ with $\alpha = 1$, 1.3, 1.6 and 2.0. Increasing the daily food ratio with $\alpha = 1.6$ resulted in an improved growth rate of the rotifers of 85% in an 8-day culture trial (Fig. 4). During this 8-day culture trial a SGR of 0.30 was measured.

Following the same approach to determine the optimal feeding rate for the experimental diet, the standard feeding regime $CSH = \beta 0.027D^{0.415}V$ was used with $\beta = 1.0$, 1.2, 1.3 and 1.4. By optimising the feeding regime from $\beta = 1$ to $\beta = 1.3$ a 170% increase in the rotifer density could be obtained in the recirculation system (RC 500) (Fig. 4). With this feeding regime a rotifer density of 8000 individuals per milliter was reached after 8 days (i.e. SGR = 0.35).

Table 3
Mean values and S.D. of the rotifer density (individuals ml^{-1}) obtained in a batch and a recirculation system with a standard feeding regime, $CS = 0.0168D^{0.415}V$[a]

Day	Batch (specific growth rate)			Recirculation (Specific growth rate)		
	Day 0–4	Day 4–8	Day 8–11	RC 100%	RC 300%	RC 500%
0	250			250	250	250
1	256 ± 21^{a} (0.02)			384 ± 35^{b} (0.43)	357 ± 50^{b} (0.36)	210 ± 70^{a} (−0.17)
2	462 ± 42^{a} (0.31)			555 ± 175^{a} (0.40)	523 ± 92^{a} (0.37)	463 ± 81^{a} (0.31)
3	506 ± 34^{a} (0.24)			677 ± 99^{a} (0.33)	613 ± 157^{a} (0.30)	513 ± 50^{a} (0.24)
4	654 ± 45^{a} (0.24)	250		706 ± 149^{a} (0.26)	873 ± 83^{a} (0.31)	747 ± 176^{a} (0.27)
5		219 ± 30 (−0.13)		911 ± 200^{a} (0.26)	1160 ± 30^{a} (0.31)	807 ± 72^{b} (0.23)
6		342 ± 26 (0.16)		1051 ± 173^{a} (0.24)	1443 ± 83^{b} (0.29)	1160 ± 0^{c} (0.26)
7		666 ± 57 (0.33)		1332 ± 227^{a} (0.24)	1877 ± 203^{b} (0.29)	1223 ± 247^{c} (0.23)
8		867 ± 60 (0.31)	250	1335 ± 194^{a} (0.21)	2203 ± 376^{b} (0.27)	1497 ± 116^{a} (0.22)
9			210 ± 33 (−0.17)	1606 ± 429^{a} (0.21)	2450 ± 184^{b} (0.25)	1480 ± 20^{a} (0.20)
10			366 ± 43 (0.19)	1641 ± 619^{a} (0.19)	2413 ± 50^{b} (0.23)	1447 ± 67^{a} (0.18)
11			445 ± 33 (0.19)	1761 ± 566^{a} (0.18)	2483 ± 297^{b} (0.21)	1520 ± 147^{a} (0.16)

[a] Means within the same row and followed by the same letter are not significantly different ($P > 0.05$). ( ) = Specific growth rate.

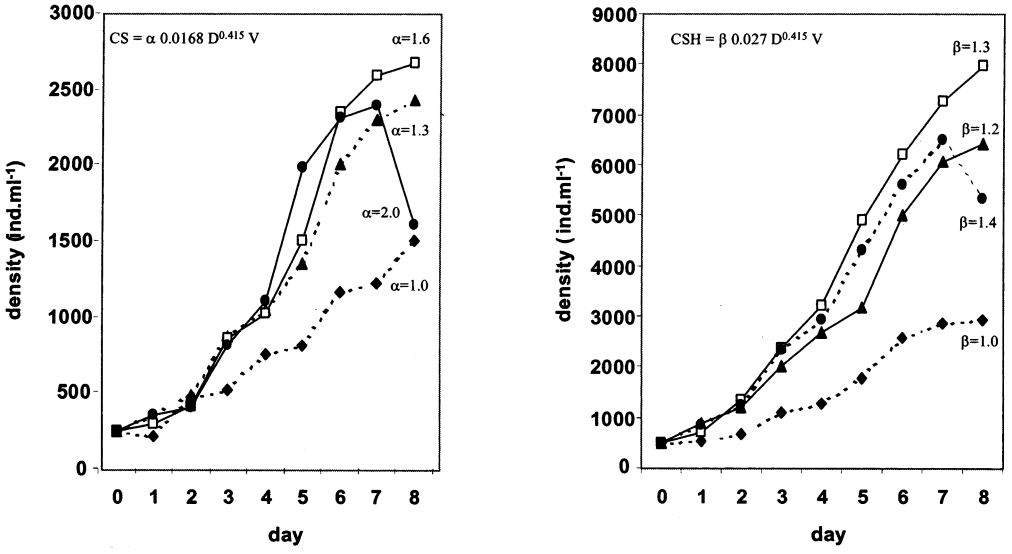

Fig. 4. Rotifer density obtained at various feeding regimes with CS® and CSH at 500% daily recirculation rate.

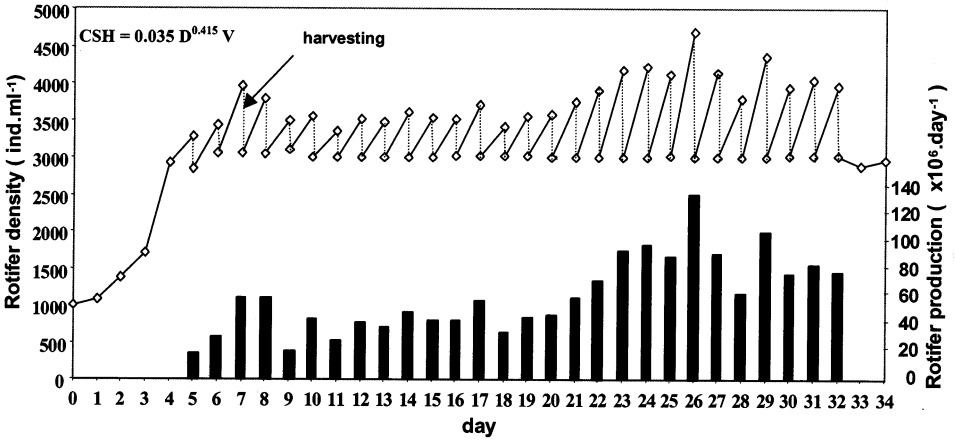

Fig. 5. Rotifer density and rotifer production obtained by daily harvest at rotifer densities above 3000 individuals per milliter with an optimised feeding scheme: $\mathrm{CSH} = 0.035D^{0.415}V$.

This standard feeding ($\mathrm{CSH} = 0.035D^{0.415}V$) was used in a continuous culture with daily harvest of rotifer. The culture was kept at the density of 3000 individuals per milliter at a recirculation rate of 500%. The culture could be maintained during 32 days and produced 1.7×10^9 rotifers (Fig. 5). The average daily harvest was $60.5 \pm 28.5 \times 10^6$ rotifers or approximately 20% of the standing population.

3.2. Physico-chemical water parameters

Low ammonium levels were observed in the beginning of the culture period in both systems. For the batch culture, the NH_4^+ level increased during the 4 days culture period from 0 to 12 mg l^{-1} and decreased after each restocking and rinsing of the rotifers. In RC 100, the NH_4^+ level gradually increased during the culture period. Lower NH_4^+ levels were obtained by increasing the daily water exchange rate from 300 to 500% in the recirculation system. Although the NH_4^+ concentrations were slightly increased at the end of the culture period, acceptable levels of 5.0 and 2.0 mg l^{-1} NH_4^+ were observed in RC 300 and RC 500, respectively (Fig. 6).

Fig. 6 shows that at the beginning of the culture period (day 0) all culture systems had a pH level of 8.0. From day 1 onwards, RC 100 showed a lower pH than in the batch culture. By increasing the daily water exchange rate from 300 to 500% a higher pH could be maintained in the recirculation system than the batch culture. Moreover, the pH was more stable in the recirculation system than in the batch culture during the complete culture period. The pH level ranged from 7.6–8.0 (during 4 days) for the batch culture system and 7.7–8.1, 7.7–8.1 and 7.9–8.1 for RC 100, 300 and 500%, respectively during the whole culture period of 11 days.

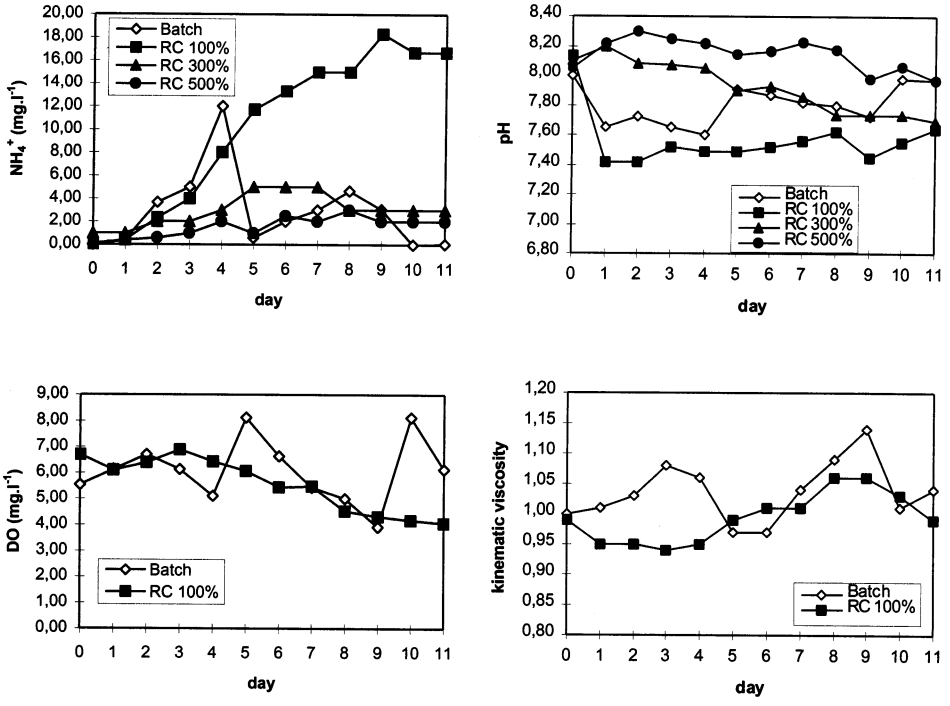

Fig. 6. The physico-chemical parameters measured in the batch and in the different recirculation systems.

Also the dissolved oxygen concentration and kinematic viscosity in the batch culture system were subjected to higher fluctuations than in the recirculation system. The protein skimmer reduced the COD from 444 mg l^{-1} in the culture water to 84 mg l^{-1}. The biofilter further reduced the COD to 2 mg l^{-1}.

During the long-term (32 days) continuous rotifer culture, low fluctuation of physico-chemical parameters were noticed. The NH_4^+ level fluctuated from 0.5 to 3.0 mg l^{-1}. The pH gradually decreased during the culture period from 7.7 at the beginning of culture period to 7.0 at the end of the culture period. Also the NO_3^- level and kinematic viscosity showed low variation during the culture period where the NO_3^- level gradually increased as a result of the nitirification process and kinematic viscosity fluctuated from 1.02 to 1.18.

4. Discussion

Using density as a parameter to evaluate rotifer performance in a batch and recirculation system it could be demonstrated that highly significant differences in the growth of the rotifer populations could be obtained with increased water exchange rates.

At increased water exchange rates the feeding scheme needed to be compensated for feed losses in the protein skimmer. Increasing the feeding rate to compensate for the losses in the recirculation system and to cover the basic needs of the denser rotifer population resulted in an improved population growth. However, this increased food consumption was marginal compared to the increased rotifer production. The use of a recirculation system with a 300% daily water exchange instead of 100% enabled the production of 1.5 times denser rotifer cultures. Raising the daily water exchange rate from 300 to 500%, only required an adjustment in the feeding scheme with $\alpha = 1.6$ and resulted in a 12% increase in rotifer production. Higher exchange rates may even give better rotifer performances but could not be investigated due to the limited water penetration through the 33-μm filter screen. In this respect it is very likely that with higher performance filter systems higher rotifer densities could still be obtained.

The application of the experimental diet (CSH) in the recirculation system resulted in an increase of 17% of the specific growth rate compared to CS®, enabling a standing population of 800 million rotifers after only 8 days after the inoculation of the system in 100 l (Fig. 4). Compared to the batch system this means a 100% gain in production time and moreover considerable savings in labour since the recirculation was not subjected to any intermediate rinsing or cleaning during the production period. This may also have advantages on the energy consumption since less water needs to be heated. Based on the results from the experiments, it could be demonstrated that for the production of a same amount of rotifers (10^9) in a batch or a recirculation system the use of the more performant diet CSH resulted in 30% lower food consumption (Table 4).

The improved water quality obtained with the recirculation system resulted in stable rearing conditions. The water quality parameters were strongly related to the

Table 4
Comparison in the food consumption and length of the culture period for different rotifer culture systems for the production of 10^9 rotifers in 100 l

	Culture system		
	Batch	RC 500%	RC 500%
Food	CS®	CS®	CSH
Food consumption (g)	1027	1046	763
Length of the culture period (days)	16[a]	11[b]	8[b]

[a] During the rearing period four restockings and rinsings need to be performed.
[b] No restocking and rinsing.

rate of water exchange in the rotifer cultures. The batch culture system showed higher ammonium concentrations than the recirculation systems. Those conditions are attributable to the capacity of the system itself, which is not able to carry away the accumulated dissolved and suspended organic matter from the system (e.g. rotifer feces, flocules and diet). Increasing the daily water exchange rate in the recirculation system resulted in much better and more stable water quality parameters. This is the result of a better removal of the particles (more passages through the protein skimmer) and a better exposure to the biofilter. In this respect the protein skimmer was the most efficient tool in removing organic compounds since it was responsible for a drop of 81.2% in the chemical oxygen demand (COD).

Batch rotifer culture systems are usually rinsed and restocked after approximately 4 days when 10 mg l^{-1} NH_4^+ is reached and the rotifer growth is declining. Since the toxicity of un-ionised ammonia (NH_3-N) released from ammonium (NH_4^+-N) is a function of the pH (Losordo and Westers, 1994), it is common practice to reduce the pH in high rotifer density batch cultures (Yoshimura et al., 1996). In the batch culture 2.2% (0.28 mg l^{-1}) is present as un-ionised ammonia at the end of each 4-day culture cycle. In the recirculation system (500%) the un-ionised ammonia reaches only 0.09 mg l^{-1} at the end of the rotifer culture cycle (i.e. after 11 days). It is obvious from these numbers that, although the recirculation system is operating at a higher pH than the batch system, the concentration of un-ionised ammonia is lower at each moment even without pH adjustment. Although no negative influence of the pH has been reported on the growth of the rotifers it may be sensible to keep higher and better buffered rearing systems around the normal pH of seawater. Especially, when the rotifers are meant to be fed to predators this may have many advantages. The subsequent rinsing before feeding them to the predators and the immersion in the larval tank can occur without major changes in water quality that can only benefit in a better quality of the larval diet.

Although high rotifer densities may be attractive to farmers, it may be dangerous to run operations close to their maximum carrying capacity. The continuous production of rotifers at lower densities by daily harvests offers better perspectives since it can be maintained for longer rearing periods. Since the system is not overloaded it is also less subjected to fluctuations in physico-chemical parameters.

This was reflected in reliable daily productions of $\approx 20\%$ of the standing crop and long production cycles.

In conclusion, it can be stated that a lot of disadvantages inherent to the batch culture system can be solved by the use of a recirculation system. The use of such a recirculation system has proved to reduce labour cost, water consumption and heating cost while ensuring stable physico-chemical parameters. Although no bacterial counts were performed on the system as of now, it is not unlikely that also from a microbial point of view more stable culture conditions could be obtained, which could also avoid the incidence of unexplained crashes of rotifer populations in batch cultures.

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