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Rainbow trout (*Oncorhynchus mykiss*) fry initiation in closed water recirculation systems¤

Iniciación de alevinos de trucha arcoíris (Oncorhynchus mykiss) en sistemas cerrados de recirculación de agua

Iniciação de alevinos de truta arco-íris (Oncorhynchus mykiss) em sistemas fechados de recirculação de água

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Summary

Background: rainbow trout is widely accepted for human consumption worldwide, however, its culture requires large quantities of high quality water**. Objective**: the aim of this study was to evaluate growth and survival of rainbow trout fry cultured in closed water recirculation systems (CRS). **Methods**: three sequential experiments were conducted, each with three CRS consisting of a fish tank, a gravel bio-filter, a water pump and aeration pumps. Fifty rainbow trout fry were placed in each system and fed with 45% protein pellets. Physicochemical and fish growth parameters were measured for each system at days 1, 15, and 30. **Results**: water parameters were maintained within normal values for the species in all the experiments. Observed fish growth was similar or greater than previously reported. Survival was lower, mainly due to the presence of Ich in some of the cultures. Although total biomass increase was small due to the low stocking density, condition factor and food conversion were within reported values. **Conclusion**: this study demonstrates that simple recirculation systems with a very small amount of water can be used for rainbow trout initiation.

Key words: *aquaculture, biofiltration, fish growth, sustainable development, water use.*

Resumen

Antecedentes: la trucha es una especie de consumo de amplia aceptación a nivel mundial. Sin embargo, su cultivo requiere de la utilización de grandes cantidades de agua de muy buena calidad. **Objetivo**: el principal objetivo de este trabajo fue el de evaluar el crecimiento y supervivencia de alevinos de trucha

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arcoíris cultivados en sistemas cerrados de recirculación de agua (CRS). **Métodos**: se llevaron a cabo tres experimentos secuenciales, cada uno de ellos con tres CRS que estaban compuestos de un tanque de peces, un biofiltro en grava, una bomba de agua y bombas de aireación. En cada sistema se sembraron 50 alevinos de trucha arcoíris que se alimentaron con concentrado al 45% de proteína. Los días 1, 15 y 30 de cultivo se tomaron parámetros fisicoquímicos del agua y parámetros de crecimiento de los peces para cada sistema. **Resultados**: en general, en todos los experimentos se mantuvieron parámetros del agua dentro de los reportados para la especie. El crecimiento fue similar o mayor a lo observado en otros trabajos. La supervivencia fue menor, debido principalmente a un brote de Ich en algunos cultivos. El incremento en biomasa total fue bajo ya que la densidad de siembra fue baja; el factor de condición y la conversión alimenticia fueron similares a valores ya descritos. **Conclusión**: de acuerdo con los resultados obtenidos en este trabajo, los sistemas de recirculación simples pueden ser utilizados para la iniciación de trucha arcoíris, con un nivel de utilización de agua muy bajo.

Palabras clave: *acuicultura, biofiltración, crecimiento de peces, desarrollo sostenible, uso del agua.*

Resumo

Antecedentes: a truta é uma espécie de ampla aceitação a nível mundial. No entanto, o seu cultivo requer a utilização de grandes quantidades de água de boa qualidade. **Objetivo**: avaliar o crescimento e a sobrevivência de alevinos de truta arco-íris cultivados em sistemas fechados de recirculação de água (CRS). Métodos: Foram realizados três experimentos sequenciais, cada um com três CRS. Os CRS estavam constituídos por um tanque com peixes, um biofiltro em cascalho, uma bomba de água e bombas de aeração. Em cada sistema foram cultivados 50 alevinos de truta arco-íris que se alimentaram com uma ração de 45% de proteína. Os dias de cultivo 1, 15 e 30 foram medidos os parâmetros físico-químicos da água e os parâmetros de crescimento dos peixes em cada sistema. **Resultados**: em todos os experimentos, os parâmetros da água estiveram dentro dos reportados para a espécie. O crescimento foi semelhante ou maior ao observado em outros estudos. A supervivência foi menor devido ao surto de Ich em alguns cultivos. O incremento em biomassa total foi baixo porque a densidade de alevinos também foi baixa. O fator de condição e a conversão alimentar foram semelhantes aos valores descritos para a espécie. **Conclusão**: de acordo com os resultados deste trabalho, os sistemas de recirculação simples podem ser usados para a iniciação da truta arco-íris com um menor consumo de água.

Palavras chave: *aquicultura, crescimento de peixes, biofiltração, desenvolvimento sustentável, uso da água.*

Introduction

Rainbow trout (*Oncorhynchus mykiss*) presence in Colombia dates to the 1930s when it was first introduced to the country. This species' production in the country increased rapidly from 1,256 tons in 1986 to 51,376 tons by 1999 (Salazar, 2001). However, production decreased from approximately 1,931 tons to 2,253 tons by 2002 (Salazar, 2002). By 2007, national fishery productivity estimates reached 46,267 tons, with trout production representing 2% of this total. Regional contributions from the same year were as follows: Antioquia: 90.99 tons; the Coffee region, Tolima, and Cundinamarca: 226.79 tons; Santander: 95.22 tons; all other country regions: 651.72 tons (Aldana *et al*., 2007).

Trout production in raceway systems uses culture densities ranging from 1.8 Kg/Lpm to 9.6 kg/Lpm (Stickney, 2000). Water temperature ranges between 10 ºC and 18 ºC, with pH values from 6 to 8, and a minimum oxygen level of 4 to 5 mg/L. These aquaculture systems are typically located at high altitude and in some damp water systems in Cundinamarca and Boyacá provinces (Ustate, 2002). Great amounts of high quality water are commonly used to avoid water deterioration, which would easily lead to outbreaks of sanitary problems (Cain and Garling, 1993). Therefore, there is a great interest in developing and adapting alternative strategies to diminish the hydric overexploitation issues of current production systems. The use of closed water recirculation systems (CRS) is one of these strategies. CRS can be installed at any location, allowing workers to easily access the fish for feeding, monitoring, and system management, better control of environmental conditions (Temperature, pH, dissolved oxygen, ammonia, nitrite), and improved fish production per volume of water. CRS use only 5% of the water required by other systems to achieve similar fish production, hence saving a significant amount of water. Additionally, CRS can function partially independent from the weather (Losordo *et al*., 1992; Dunning *et al*., 1998; Sirakov and Ivancheva, 2008; Emparanza, 2009; Roque *et al*., 2009; Good *et al*., 2010; Davidson *et al*., 2011). However, these systems have a high initial cost, depend on electricity, and require qualified personnel.

Taking these considerations into account, we aimed to evaluate growth and survival of rainbow trout (*O. mykiss)* fry in CRS, mathematically modeling fry growth and determining several crucial productivity parameters for recirculation systems.

Materials and methods

The study took place in the Aquaculture Laboratory of the Estación Experimental Rio Grande, Cajicá, at the Universidad Militar Nueva Granada. To build each CRS, we used a 500 L plastic tank (Colempaques, Bogotá, Colombia) and created a 2.25 cm diameter opening 5 cm above the bottom of the tank for drainage. Inside this tank, a water pump (Resun® SP 2500 L, 40W, output 1400 L/h, Resun, Shenzhen, China) connected to a 2.54 cm diameter and 1.30 m length hose was used to transport water to the bio-filter. Additionally, two Elite 802 E of 2000 cc/minute (Rolf C Hagen, Castleford, United Kingdom) exit air pumps ventilated the system. The bio-filter was made of a 90 cm height and 50 cm diameter tank to which a 93 cm long and 2.54 cm diameter PVC pipe was adapted in order to transfer water to the fish tank. The bio-filter had three filtration layers as follows: first, a 22.5 cm layer of gravel (2 to 3 cm diameter), a second layer of aquarium gravel (0.5 to 1 cm diameter) totaling up to 40 cm, and a third layer of 250 g activated charcoal wrapped in 50 cm^2 plastic mesh to complete the bio-filter. All bio-filter materials, except the activated charcoal, were washed for two days with 100 L of 4% hypochlorite aqueous solution and rinsed. Nitrifying bacteria inoculation was performed with a biological supplement (Nutrafin Cycle®, Rolf C Hagen, Castleford, United Kingdom), consisting of 10 ml per each 35 L of water prior to fish introduction to the CRS. The entire system was run for 2 weeks to promote water maturation. By the end of the 2 weeks, the fry were introduced to the system and the tank was covered with a 45% mesh to restrict fish from escaping. Fish were maintained under a 12 h: 12 h (day: night) natural photoperiod.

On a weekly basis the bottom of the tank was cleaned, the ventilation provided by the air pumps was inspected, and pipe and hoses were checked to eliminate debris and de-obstruct air diffusion. Fish were visually inspected for symptoms of disease or pathogen presence. The exchange of 25% of water was conducted biweekly to eliminate excessive dissolved solids, while 200 g NaCl/system was added to prevent any disease outbreaks.

Fish were obtained from a commercial fish distributor, considering the features reported by Ebeling *et al. (*1995) and Stevenson (1999). Average initial fish weight was 0.67 ± 0.18 g (Experiment A), 0.47 ± 0.12 g (Experiment B), and 0.64 ± 0.19 (Experiment C). Fish were fed three times per day (7:30, 10:30, and 13:30) using commercial fish feed with 50% protein for the initial stage and subsequently adjusted to the 10% of average individual fish weight. Adjustments to provide feed were done biweekly taking into account the data obtained during each weight sampling.

A total of 150 fish were used for the first experiment (Experiment A), equally distributed in three replicas (1, 2, 3). Fish were maintained for 30 days in each system. By day 30 fish were removed and all CRS components were cleaned and disinfected before subsequent experiments began (first B, and then C).

Initial variables were recorded in sampling (1) followed by two biweekly samplings (2 and 3) using 10 individuals randomly selected from each replica and group. Weight (W), total length (TL), and standard length (SL) for each group were obtained with an analytical balance (Model KERN ALS 120-4N) and a Vernier caliper, respectively. The following physicochemical parameters were evaluated biweekly using freshwater analysis kits: ammonium, nitrite, pH, and general hardness of the water. The percentage of non-ionized ammonium was calculated considering pH and temperature (Masser *et al.*, 1999) (Nutrafin Aquarium Test for Fresh Water; Canada: ROLF C: Hagen INC, Montreal, Qc H4R 1E8). Temperature and pH were measured with a potentiometer.

The following equations were used to estimate productivity parameters:

Weight gain (Mercado, 2006): WG = (Average final weight (g) – Average starting weight (g)).

Growth specific rate (GSR) (Alvarado, 1999): GSR = 100 * [(Ln Final weight (g) – Ln Starting weight (g))/t], where time (t) is measured in days.

Absolute growth rate (AGR) (Arce and Figueroa, 2003): AGR = (Final weight (g) – Starting weight (g)) / (T2 - T1), where T2 is total days of culture and T1 is the starting time in days.

Condition Factor (K) (Alvarado, 1998; Bastardo and Sofía, 2003; Morales, 2004): $K = (W/L^3) * 100$, where W is average weight (g) and L is average length (cm).

Food conversion factor (FCF) (Zapata *et al.*, 2008; Morales, 2004; Alvarado, 1999): FCF = FC / WG, where FC is feed consumed (g) and WG is weight gain (g).

Survival (S) was determined as the difference between the number of initial and final individuals (Pineda, 1999): S = (FNf/FNi)*100, where S is survival percentage. FNf and FNi are final and initial fish number, respectively.

Weight, total length, and standard length data were tested for normality (Shapiro and Wilk). When data had a normal distribution, a variance analysis (ANOVA) was performed followed by an HSD Tukey test to identify growth (weight, total length, and standard length) and productivity differences among experiments. A 5% ($p<0.05$) significance level was used (Zar, 1999). Three growth models were tested (linear, exponential and potential) taking the previous data into account. All statistical tests and analyses were completed using the R software (version 2.8.1).

Results

Ammonium, nitrite, pH, general hardness of the water, and temperature data were recorded for each system (Table 1).

Growth data are reported in table 2. Significant fish size and weight increments were observed for all three experiments conducted in this study. Although the specimens of each experiment began with different size and weight values due to differences by lot, similar growth tendencies were present. The R^2 values (Table 3) displayed a better adjustment to the exponential growth model in fives cases and to potential growth model in four cases.

Productivity data parameters are shown in table 4. Obtained values were found to be between the previously reported productivity ranges for the species (Blanco, 1994; Chiodo, 1999; Stevenson, 1999; Stickney, 2000).

Table 1. Physicochemical parameters for the three experiments. Sampling (1) corresponds to initial values for each water parameter evaluated.

Experiment	Replica	Sampling	Total Ammonium mg/L	Non-ionized Ammonium mg/L	Nitrite mg/L	General Hardness mg/L	pH	T °C
A	1	1	0	0	0	40	7.84	16.4
		2	0.1	0	0.1	40	6.96	15.7
		3	0	0	0	20	5.35	16.9
	2	1	0	0	0	40	7	16.4
		2	0.1	0	0.1	40	6.19	15.7
		3	0.6	0	0.1	60	5.3	16.8
	3	1	0	0	0	20	7.14	16.4
		2	0.1	0	0.1	40	6.18	15.7
		3	0	0	0.1	20	5.36	16.9
B	1	1	0	0	0	20	7.18	15.4
		2	0	0	0.1	20	6.6	16.5
		3	0	0	0.3	40	6.15	16.6
	2	1	0	0	0.1	20	6.86	15.4
		2	0	0	0.1	40	6.6	16.5
		3	0.6	0	0.1	20	5.97	16.6
	3	1	0	0	0	20	6.96	15.4
		2	0	0	0.1	20	6.6	16.5
		3	0.6	0	0.1	40	5.38	16.6
C	1	1	0.6	0	0	20	6.94	16.6
		2	0.6	0	0.1	40	6.2	17
		3	0.6	0	0	40	6	16
	2	1	0.6	0	0	20	6.8	16.6
		2	0.6	0	0.1	40	6.46	17
		3	0.6	0	0	20	6.1	16
	3	1	0.6	0	0	20	6.8	16.6
		2	0.6	0	0	20	5.59	17
		3	0.6	0	0.1	40	6	16

Table 2. Growth data for the three experiments.

		Follow-up (weekly)		
Variable	Experiment	1	2	3
Weight	A	0.67 ± 0.18aA	2.04 ± 0.71aB	4.55 ± 1.71aC
	B	0.47 ± 0.12bA	1.21 ± 0.30bB	3.34 ±1.01bC
	C	0.64 ± 0.19aA	1.94 ± 0.68aB	3.34 ± 0.99bB
Standard Length	A	3.66 ± 0.28aA	4.83 ± 0.65aB	6.27 ± 0.87aC
	B	2.99 ± 0.18bA	3.90 ± 0.43bB	5.36 ± 0.64bC
	C	3.40 ± 0.37cA	4.70 ± 0.63aB	5.51 ± 0.63bB
Total Length	A	4.15 ± 0.28aA	5.32 ± 0.66aB	7.14 ± 0.82aC
	B	3.47 ± 0.19bA	4.40 ± 0.45bB	6.01 ± 0.75bC
	C	3.90 ± 0.38cA	5.33 ± 0.65aB	6.35 ± 0.68bC

Significant differences ($p<0.05$) between experiments in each sampling (a, b, c). Significant differences ($p<0.05$) within experiment per sampling (1, 2, 3).

Table 3. Mathematical growth models obtained for weight, standard length, and total length, related to time (samplings).

Variable	Experiment	Model with best adjustment	R^2	Equation
Weight	A	Potential	0.800	$Y=0.634x^{1.676}$
	B	Exponential	0.876	$Y=0.171e^{0.870x.}$
	C	Potential	0.802	$Y=0.620x^{1.510}$
Standard length	A	Exponential	0.758	$Y=2.799e^{0.266x}$
	B	Exponential	0.835	$Y=2.221e^{0.288x}$
	C	Potential	0.726	$Y=3.399x^{0.244}$
Total length	A	Exponential	0.805	$Y=3.138e^{0.269x}$
	B	Exponential	0.821	$Y=2.617e^{0.212x}$
	C	Potential	0.762	$Y=3.886x^{0442}$

Mathematical growth models with high determination coefficient (R^2) obtained for weight related to time (samplings). Capital letters indicate the corresponding experiment.

Table 4. Productivity parameters in the three experiments.

Experiment	Starting Total Biomass (g)	Final Total Biomass (g)	WG (indv) (g)	GSR (% day)	AGR (g/day)	K(f)Factor	K(i)Factor	FCF	% Survival
A	33.85±2.39a	212.15±93.63a	3.87±1.58a	6.15±1.13ab	0.13±0.05a	1.20±0.16b	0.95±0.05c	1.17±0.42a	83.33±18.90a
B	23.68±2.38b	139.67±19.78b	2.87±0.12b	6.52±0.32a	0.07±0.04b	1.54±0.04a	1.13±0.11a	1.43±0.14a	91.33±4.16a
C	32.17±3.52a	128.67±26.24a	2.71±0.02c	5.50±0.27a	0.09±0.01b	1.31±0.03b	1.08±0.03b	0.88±0.08b	76.00±3.46b

Significant differences ($p<0.05$) between experiments A, B, and C (a, b, c).

Discussion

Physicochemical parameters

In general, water quality parameters remained within the permissible ranges suggested for *O. mykiss* (Klontz, 1991; Losordo *et al.*, 1992; Blanco, 1994; Ebeling *et al*., 1995; Chiodo, 1998; Masser *et al*., 1999; Stevenson, 1999; Stickney, 2000). Although this study was not designed to define the loading capacity of CRS, our data suggest that the maximum limit was not reached as the physicochemical parameters were maintained within the reported range for the species. However, we did find that ammonium values behaved differently for experiment C, being higher than in the other experiments. We attribute this difference to the stage of tank maturation by the time the fish were introduced to the system. In spite of the increased total ammonium level found in experiment C, the non-ionized ammonium generated by the system at the established pH level did not alter growth. We also observed some acidification tendency in the system. Starting pH values were around 7, while ending values ranged between pH 5 to 6. The observed pH changes are expected for these culture systems, leading to keep the non-ionized ammonium levels low thereby benefitting the system.

Growth

In this study, the organisms had higher weight increments compared with other studies (Ceballos and Velázquez, 1988), where *O. mykiss* individuals were maintained at 600 fish/m^3 for 84 days with 0.3 g and 4.5 g as initial and final weight, respectively. Our weight increments data for the three experiments (A, B and C) suggest fast growth occurred during the evaluated culture period. The growth curve proposed by Blanco (1994) indicates the average weight would be approximately 5 g after 60 days, which is the final average weight reached in our work after only 30 days. Total length reached values higher than 5.95 cm in all replicas from all three experiments, similar to the report by the Peruvian Vice-Ministry of fishery (2004), where a length of 4 cm corresponds to a weight of 0.72 g. However, the similarities with that study diminished when they reported length of 2.5 g for 6 cm while we obtained heavier animals within the 6 to 7 cm size range. Regarding the mathematical growth models, our data correspond more accurately to the exponential and potential models as is expected for young organisms in their early growth phase.

Productivity parameters

According to the increment in total gross biomass, the three experiments had increments greater than 100 g for a 30-day period. Rosado and Erazo (2001) and Merino (2005) advise that biomass range should be between 3 to 8 kg/m^3, which is significantly higher than the maximum density obtained in this study (0.26 kg/0.75 m^3 or 50 fish/0.75 m^3) in Replica 1 (experiment A). Here we have only considered the organismal beginning and we do not know yet the load capacity of the system. However, our preliminary data using common carp (*Ciprinus carpio*) allowed us to reach up to 24 kg/m^3. Although common carp is more rustic than rainbow trout, physicochemical water parameters and growth data in this study indicate that we have not reached the system limits yet. Thus, it is possible to significantly increase density as well as culture duration, allowing us to maintain a greater number of *O. mykiss* individuals until they reach larger sizes resulting in a higher total biomass and reduced production cost (tanks, air pumps and electricity). This is highly relevant taking into account the amounts of water traditionally used in trout cultures. For example, the water flow used for rising trout fry range between 5 to 70 L/min in channel systems (Al-Hafedh *et al.*, 2003; Merino, 2005; Szyper *et al.*, 2005; López *et al.*, 2007) indicating that in a 30 day period with constant entrance and exit flow, the water volume in channel systems ranges from 216,000 to 3,000,000 L/month, which is significantly higher than the 1,500 L/month required by CRS used in this study, including all water exchanges made during the experiments.

Regarding GSR, the values expressed in percentage/day are higher than 5% in all tested groups. The GSR values obtained in our study are superior to the ones obtained by other authors using cage and channel systems: 3.35% (Morales, 2004), 1.76% (Alvarado, 1999), and 1.70% (Bastardo and Sofía, 2003). Cumulative growth rate was higher than 0.7 g/day in all experiments except for replica B2, which showed the lowest AGR (0.02 g/day). The AGR values found in this study were lower than those obtained by other researchers (Vergara *et al.*, 1998). We consider the differences to be due to the developmental stage of the individuals used in this study (juveniles with 53.0 g initial weight). The final condition factor (Kf) increased for all replicas. Our results were higher than the ones obtained for cage systems: 1.08 (Alvarado, 1998), 1.34 (Morales and Quirós, 2007), 1.1 (Bastardo and Sofía, 2003). According to Cain and Garling (1993) the values obtained in our experiment correspond to properly fed fish, which are converting the consumed food into gross biomass. Relative to the experiment FCF values, our data show a good conversion compared with what has been reported for raceway culture systems (Ceballos and Velázquez, 1988). Survival in Experiment A was 83.33%, which may have been greater if there not been an Ich (*I. multifiliis*) outbreak in one of the replicas. The average survival percentage in this study was 83.56%, which is lower than other reports: 97.17% (Alvarado, 1999) and 85% (Ceballos and Velázquez, 1988). However, it is important to mention that a good proportion of the mortality found in CRS described here was associated with fish jumping out of the systems. We believe that using a mesh system would increase survival to more than 90%.

We conclude that CRS use significantly reduces water consumption and maintains water quality, resulting in good productivity parameters for *O. mykiss*. Thus, the next step will be to define the CRS load capacity to increase it and determine if a simple CRS design could be an economically and environmentally sustainable alternative for rainbow trout culture in Colombia.

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