Evaluation of a recirculation system for Dorada (*Brycon moorei*-Steindachner 1878) larvae in the Magdalena River^{*}

Evaluación de un sistema de recirculación para la etapa de larvicultura de dorada (<u>Brycon moorei</u>-Steindachner 1878) del Río Magdalena

Avaliação de um sistema de recirculação para a fase de larvicultura de dourada (<u>Brycon moorei</u>-Steindachner 1878) de Rio Magdalenao

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Abstract

Recirculating aquaculture systems allow increased productivity during larval stages due to a greater control of water quality parameters. Dorada larvae (*Brycon moorei*) were used 12 hours post hatching (HPE) to evaluate three planting densities with four replicates per treatment and 10, 20 and 30 larvae L-1 (T1, T2 and T3, respectively). The larvae were fed with Bocachico (*Prochilodus magdalenae*) larvae, *Artemia salina* nauplii and a 40% crude protein (CP) commercial diet for 15 days. Results showed significant differences between treatments (p<0.0001) for weight gain and specific growth rate (TCE), where T1 performed better (73.24±19 mg and 14.21±5.02%, respectively); followed by T3 (67.15±24.3 mg and 13.94±7.51%) and T2 (55.67±18.33 mg and 13.36±5.75%). Survival rate was inversely related to density, with highly significant difference (p<0.0001)(39.37±6.84%, 28.00±2.80% and 14.87±6.47%) for T1, T2 and T3, respectively. Simultaneously, cannibalism was directly related to planting density. The highest profitability was obtained with T2.

Key words

Brycon moorei, density, larval rearing, recirculation survival.

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Resumen

Los sistemas con recirculación para acuicultura permiten el incremento de la productividad en la etapa de larvicultura, debido al mayor control de los parámetros de calidad de agua. Larvas de dorada (*Brycon moorei*) de 12 horas post eclosión (HPE) fueron utilizadas para evaluar tres densidades de siembra con cuatro replicas por tratamiento, 10, 20 y 30 larvas L-1 (T1, T2 y T3, respectivamente). Las larvas fueron alimentadas durante 15 días, con larvas de bocachico (*Prochilodus magdalenae*), nauplios de *Artemia salina* y una dieta comercial de 40% de proteína cruda (PC). Al final del experimento, los resultados muestran diferencias altamente significativas entre los tratamientos (p<0.0001) para ganacia de peso y tasa de crecimento específica (TCE), obteniendo un major desempeñoen T1 (73,24±19 mg y 14,21±5,02%, respectivamente); seguido por T3 (67,15±24,3 mg y 13,94±7,51%) y T2 (55,67±18,33 mg y 13,36±5,75%). La sobrevicencia se relacionó de manera inversa con la densidad con diferencia altamente significativa (p<0.0001) (39,37±6,84%; 28,00±2,80% y 14,87±6,47%) para T1, T2 y T3, respectivamente y, simultáneamente, el canibalismo fue directamente relacionado con la densidad de siembra. Por otro lado, la mayor rentabilidad fue obtenida con T2.

Palabras clave

Brycon moorei, densidad, larvicultura, recirculación, sobrevivencia.

Resumo

Os sistemas de recirculação de aquicultura permitir o aumento da produtividade na fase de larvicultura devido ao melhor controle dos parâmetros de qualidade da água. Larvas Bream (*Brycon moorei*) 12 horas após a eclosão (HPE) foram utilizados para avaliar três densidades de estocagem, com quatro repetições por tratamento, 10, 20 e 30 larvas L-1 (T1, T2 e T3, respectivamente). As larvas foram alimentadas durante 15 dias com bocachico larvas (*Prochilodus magdalenae*), náuplios *Artemia salina* e uma dieta comercial de proteína bruta de 40% (CP). No final da experiência, os resultados mostraram diferenças altamente significativas entre tratamentos (p < 0,0001) para o ganho de peso e taxa específica de crecimento (TEC), obtendo-se um grande desempeño en T1 (73,24±14,21 mg e 19±5,02%, respectivamente); seguido de T3 (67,15±24,3 mg e 13,94±7,51%) e T2 (55,67±18,33 mg e 13,36±5,75%). O sobrevicencia está inversamente relacionada com a densidade, com diferença altamente significativa (p<0,0001) (39,37±6,84%; 28,00%±2,80 e 14,87±6,47%) para T1, T2 e T3, respectivamente e, simultaneamente, o canibalismo foi diretamente relacionada à densidade de estocagem. Por outro lado, o maior rendimento foi obtido com T2.

Palavras-chave

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Brycon moorei, densidade, larvicultura, recirculação, sobrevivência.

Introduction

The Brycon genus includes more than 60 species of fresh water fish, and can be found distributed in Central and South America¹⁶. Doradas, as representatives of such group, are stenohaline and important species as commercial fish in the Magdalena and Sinú rivers, in Colombia. They have been reproduced by decades, by the action of researchers and aquaculturers, because of their size, rapid growth, omnivorous feeding²², acceptance of artificial food, from both vegetal and animal origins, a culture of consumption of their meat and their character as excellent sport fish^{4,5,23}. There are, nevertheless, evidences of the fact that this fish group is in high jeopardy²⁰. Regarding the fact that most of the Colombian native fish have altricial or indirect development larvae, several authors say that the critical time and the success in the production of fingerlings is the start of the exogenous feeding. Given this fact, this stage requires a very special care^{1,4,15}. Even though a number of environmental factors such as population density, shelters, clarity of the water, light intensity and feeding frequency, among others, are linked to the larval development, the availability and the quality of the food seem to be the most important factors to be regarded for larviculture activities¹¹.

The use of recirculation systems in the larviculture stage has had, as a result, the industrialization of pisciculture in several countries, with species such as the Atlantic halibut, *Hipoglossus hipoglossus*²⁹, the sea bass *Dicentrarchus labrax*²⁵, the African catfish, *Clarias gariepinus*¹³, the Indian rohu, *Labeo rohita*²⁸ and the tiger sorubim, *Pseudoplatystoma corruscan*²¹. This technological development has made possible an increase of the efficiency and the profitability of fish industries, with environmental sustainability as an added value, and the use of such techniques in the conservation of native species due to the re-population and the reduction of the capture pressure given by overfishing and by the environmental damage.

In Colombia, the information about larviculture in native species from warm waters in super intensive systems is scarce or inexistent. Therefore, the objective of this research work is to evaluate the larviculture stage of the *Brycon moorei* (Steindachner 1878), in a recirculation system, with three containment densities, and its effect on the growth and survival of the fish and on the profitability of the system.

Materials and methods

Aval Ethics Committee for animal experimentation

Act number 001 September 2012. The experiment is framed within the principles of animal welfare. Ethics Committee of the Corporación Universitaria Lasallista.

Location

The Research work was performed in the pisciculture station of Universidad de Antioquia, located in San José del Nus, San Roque, at 735 meters above the sea level and at an average temperature of 24 °C.

Biological Material

Two male individuals were chosen given their external maturity characteristics $(1,56\pm0,23 \text{ kg})$ and a female (2,7 kg), to which an ovarian biopsy by cannulation was applied. A hormonal induction protocol was used. It was a modification of the hypophization protocol standardized by Woynarovich & Horvath³⁰. The fertilization of the eggs was performed in a semi-natural way. They were collected and incubated in volumes of 2 L of hydrated egg per incubator (an up flow 60 L incubator was used, with a flow rate of 3,5 L min-1). The hatching percentage at 12 hours after the fertilization at 24,3±0,7 °C was registered at $84,3\pm1,7\%$. At the hatching time, the larvae weighed 0,76±0,8 mg. 12 hours after the hatching, the larvae were counted and randomly distributed into three treatments (10, 20 and 30 larvae L-1) with four replicas for each one, for 800, 1600 and 2400 larvae per container, respectively, until the 15th day after the hatching.

Characteristics and management of the recirculation system

The 2650 L recirculation system, explained in figure 1, was made with 16 circular plastic containers with an effective volume of 80 L. The filtration system for solid particles consisted of two bag filters of 60 L each, with a 25 μ m mesh. They worked in parallel and discharged water into the biological filtration system, which consisted of two biofilters built in two plastic tanks, each one with 200 L of capacity. The water velocity was 18 mm s-1and the hydraulic retention time was 7,14 minutes. The tanks were connected to a 25 L min-1 UV sterilizer. The clean water was stored in a main reservoir of 500 L with

magnetic level, united to an electric pump of 1,5 HP (320 L min-1) which drove the water to an elevated 250 L tank in which it was, again, distributed to the experimental tanks. The pipelines for the water distribution was made of 1,5" PVC. The water inlet pipe to drive the water to the culture tanks was made of 0,5" PVC, and the outlet pipe from the culture tanks was made of PVC 1", carrying waste water to a PVC collection pipe of 3", which ended in the filters for solid particles.

The flow used in the experimental units was 3,5 L min-1 and the hydraulic retention time was 22,85 min. The water used for the system came from an auxiliary de1/8 HP (25 L min-1) electric pump installed on the water collection channel of the pisciculture farm. 250 L of water were re-changed every day, which is equivalent to 9,43% of the total volume of the system, in order to keep the water's quality.

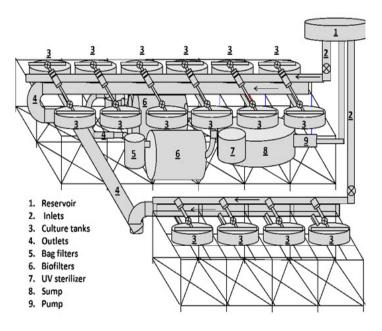


Figure 1. Schematic presentation of the recirculation aquaculture system.

Water quality

The physical and chemical characteristics of the recirculated water were registered every day, before the daily re-change, with the water quality kit for pisciculture¹ and were kept within the following ratios: temperature, 26,2±0,9 °C; dissolved oxygen, 6,75±0,71 mg L-1; pH, 7,61±0,14; thickness, 70,54±6,05 mgL-1 CaCO₃; alkalinity, 72,68±7,92 mg L-1 CaCO₃, total ammonium, 0,36±0,1 mg L-1 and non-ionized ammonium, (NH₃) 0,0114±0,006 mgL-1.

Feeding

The beginning of the exogenous feeding took place at 24 hours after hatching (HAH) at $25,3\pm0,6$ °C and the feeding protocol used is shown in table 1.

Zootechnical parameters

Statistically representative samples of larvae from each treatment were taken, and a record of their weight at the beginning and at the end of the experiment was made by the use of an analytical balance² (sensibility 0,01 mg). Weight gains (WG) and the specific growth rate (SGR) were calculated according to the formula SGR=100(Ln Pf – Ln Pi)/(fT-iT), (14) in which Pf and Pi are the initial and final weights. Tf and Ti is the time in days, fT=final time and iT =initial time²⁷. Also, the survival for each replica was registered.

Economic analysis

In order to determine the economic feasibility of each treatment, the investment was evaluated with a



¹ Hach FF1A®, Loveland, Colo., USA

² Ohaus Explorer Pro®, NJ, USA

discontinuous cash flow, time of return of the investment, breakeven and sensibility analysis, on the base of the initial investment and a cash flow period of 10 years. The breakeven was calculated by the use of the fixed expenses (including the depreciation of the machinery and the equipment, and the cost of the capital), price per sold unit (U\$ 0,11) and the cost of each unit produced. The sensibility analysis was performed reducing the sale price of the fish (5% and 10% of reduction under it was calculated in the budget). As a complementary information, and very important for the cost structure, the management time of the system was determined, taking the maturity seasons of the reproducers as a base, which are usually between March and June.

Table 1. Feeding protocol from the beginning of the exogenous feeding to 15 days after hatching (DAH).

Feeding times								
DAH	07:00	11:00	14:00	17:00				
1	LF	LF	LF	LF				
2	А	А	А	А				
3	А	А	А	А				
4	А	А	А	А				
5	А	А	А	А				
6	А	А	А	А				
7	А	А	А	А				
8	А	A+C	А	А				
9	А	A+C	А	А				
10	A+C	С	А	A+C				
11	A+C	С	A+C	С				
12	С	A+C	С	С				
13	A+C	С	С	С				
14	С	С	С	С				
15	С	С	С	С				

 $A = Brine \ shrimp$

C = commercial food tilapias 40% raw protein, sieved to 650 μ m. LF= Just hatched bocachico larvae (Prochilodus magdalenae).

Statistical analysis

The results were analyzed by the use of ANOVA with 95% of confidence. The Tukey test was used to separate the average effects at the same level of probability and a data transformation with arc-seno was performed for the survival analysis. The tests were performed with the statistic Statgraphics Centurión (XVI)® software, license supported by Corporación Universitaria Lasallista.

Results

The highest weight gain (WG) was registered for the treatment with the lowest density T1 $(73,24\pm19)$

mg), followed by the one with the highest density T3 (67,15±24,3 mg), with no significant difference (p<0.0001). The lowest weight gain was registered in the intermediate density T2 (55,67±18,33 mg) with a highly significant difference (p<0.0001) if compared to T1 and T3 (Figure 2). Similarly, when the specific growth rate (SGR) was determined, the maximum daily growth value was registered for the least dense treatment T1 (14,21±5,02% day) followed by the one with the highest density T3 (13,94±7,51% day) with no significant difference (p<0.0001). The lowest SGR was registered in the intermediate density T2 (13,36±5,75% day) with statistical difference (p<0.0001) when compared with T1 and T3.

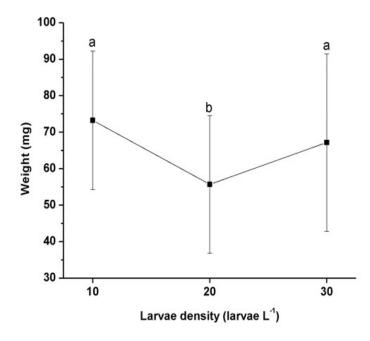


Figure 2. Relationship between weigh gain and containment density. Different letters mean a statistical difference (p < 0,0001).

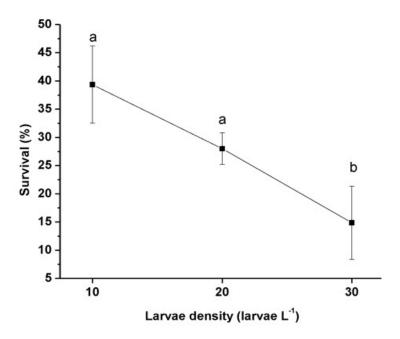


Figure 3. Relationship between density and survival percentages. Different letters mean a statistical difference (p < 0,0001).

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Table 2. Operation and maintenance costs for a recirculation system with *B. moorei* larvae at three larviculture densities (in USD).

	<i>T1</i> 12.800 larvae		<i>T2</i> 25.600 larvae		<i>T3</i> 38.400 larvae	
DIRECT COSTS						
	Cost/cycle	(%)	Cost/cycle	(%)	Cost/cycle	(%)
B. moorei larvae	\$ 35,83	9,95	\$ 71,67	16,21	\$ 107,50	20,51
P. magdalenae larvae	\$ 29,82	8,28	\$ 59,63	13,48	\$ 89,45	17,06
Brine shrimp	\$ 14,80	4,11	\$ 29,60	6,69	\$ 44,40	8,47
Food 40% RP	\$ 0,07	0,02	\$ 0,14	0,03	\$ 0,21	0,04
Sea salt	\$ 1,41	0,39	\$ 2,82	0,64	\$ 4,23	0,81
Dolomita lime	\$ 0,11	0,03	\$ 0,22	0,05	\$ 0,34	0,06
Total direct costs	\$ 82,04	22,78	\$ 164,08	37,11	\$ 246,13	46,95
INDIRECT COSTS						
Energy ¹	\$ 20,36	5,65	\$ 20,36	4,60	\$ 20,36	3,88
Quality control of the	\$ 23,33	6,48	\$ 23,33	5,28	\$ 23,33	4,45
water						
Manpower	\$ 199,93	55,51	\$ 199,93	45,21	\$ 199,93	38,14
Equipment depreciation	\$ 17,26	4,79	\$ 17,26	3,90	\$ 17,26	3,29
Capital cost (10% a year)	\$ 17,26	4,79	\$ 17,26	3,90	\$ 17,26	3,29
Total indirect costs	\$ 278,13	77,22	\$ 278,13	62,89	\$ 278,13	53,05
TOTAL COSTS/CYCLE	\$ 360,17	100,00	\$ 442,21	100,00	\$ 524,25	100,00
Young fish produced	5.039		7.168		5.710	
Unitary sale Price	\$ 0,11		\$ 0,11		\$ 0,11	
Indirect costs per unit	\$ 0,06		\$ 0,04		\$ 0,05	
direct costs per unit	\$ 0,02		\$ 0,02		\$ 0,04	
Cycles/year	6		6		6	
Annual direct costs	\$ 492,25		\$ 984,50		\$ 1.476,75	
Annual indirect costs	\$ 1.668,75		\$ 1.668,75		\$ 1.668,75	
Young fish produced a year	30.234		43.008		34.260	
Total anual costs	\$ 2.161,00		\$ 2.653,26		\$ 3.145,51	
Annual gross income	\$ 3.385,67		\$ 4.816,13		\$ 3.836,51	
Annual net nincome	\$ 1.224,66		\$ 2.162,87		\$ 691,00	
Investment return1	3,43		1,94		6,08	
Breakeven (units)	17.437		18.731		24.228	
Breakeven (sales)	\$ 1.952,65		\$ 2.097,52		\$ 2.713,07	
(-5% sale price)	\$ 1.055,38		\$ 1.922,06		\$ 499,18	
(-10% sale price)	\$ 886,10		\$ 1.681,26		\$ 307,35	

¹ Years USD = \$1786 Colombian pesos.

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The biggest survival rate was obtained in the treatment with the lowest density T1 ($39,37\pm6,84\%$) followed by the intermediate one T2 ($28,00\pm2,80\%$) with no significant statistical difference (p<0.0001). The lowest survival was obtained with the highest density T3 ($14,87\pm6,47\%$) with statistical difference (p<0.0001) if compared to T1 and T2 (Figure 3).

The data for the economic feasibility calculation of each density are shown in detail in table 2. It was determined that the highest proportion of the direct cost was food (12,83 - 26,44%) for the different treatments), followed by the cost of the larvae (9,95 - 20,51%) and the highest percentage of the indirect costs was the manpower (55,51%). The direct costs per unit produced were found between U\$ 0,02 and U\$ 0,04, in a direct correlation with the density. The indirect costs per unit produced were calculated between U\$ 0,04 and U\$ 0,06, with the lowest value for the intermediate density and the highest value for the lowest density. The investment return time was longer for the highest density (3,43 years), and the shortest was that of the intermediate density, with 1,94 years. The lowest breakeven in annual units produced was for the highest density (17.437 units), followed by the intermediate one (18.731 units) and the highest in the highest density (24.228 units).

The sensibility analysis showed a higher impact for the highest density, which means that a 5% reduction in the price would reduce the net annual income in 27,76%, while the same calculation for the lowest density was 13,82%. The intermediate density was the least affected (11,13%).

Discusión

The physical-chemical properties of the water during the experiment, in a general way, did not have great variations and are considered as normal or acceptable to cultivate fish of this genus^{6,24}.

According to several authors, the species of the *Brycon* genus have a cannibal behavior from the moment the exogenous feeding begins^{2,3,19,26}. This behavior was also registered in this work after 24 hours from the hatching moment. Within the cannibalism types, cannibalism among siblings is particularly important because it compromises the predator's development. Due to the

fact that cannibalism is modulated by hunger and by the metabolism, and is also boosted by the size differences, it is more intense among the rapid growing larvae and more common in young than in adult fish. Additionally, it starts earlier and is more acute in rapid growth tropical species than in those from temperate regions. These characteristics cause the fact that *Brycon moorei* genus shows one of the earliest and intense cannibalisms reported in fish⁸. *Brycon amazonicus* begin the exogenous feeding between 30 y 36 hours after hatching, thus starting their cannibal behavior⁷. For *Brycon moorei sinuensis* larvae, the beginning of the exogenous feeding is reported between 22 and 23 hours after hatching⁵.

Research Works about recirculation systems with tropical native species are scarce so far. For the larviculture of *Brycon amazonicus*⁹, between the densities analyzed, the most adequate stocking density in ponds to produce fingerlings was 0,12 larvae L-1. In *Brycon orbignyanus* larviculture¹⁰, the stocking density in pond was 0,09 larvas L-1. Both research works obtained a 50% survival rate. Comparing the traditional system with the production model in a recirculation system, this research work obtained its best survival and growth rates in a stocking density of 10 larvae L-1 with a 39,37% survival.

The weight variation within the different treatments is compatible with Koebele's report¹⁸, who explained that the heterogeneous growth of larvae and fingerlings is usually related to the competition for food and stress due to factors such as social interactions and hierarchy. Besides, this hierarchy directly increases as the stocking density does¹⁷. For this research work the lowest and the highest densities had a weight gain with no significant difference and a similar standard deviation. This is due to the fact that the lowest density provided a wider space to grow up and less cannibal larvae, easing the survival of a bigger proportion of small larvae, while the highest density had a bigger number of cannibal larvae, with a bigger size (2,6 times bigger than their siblings) and the larvae that consumed exclusively the food provided grew less and were almost totally devoured.

Concerning the efficiency in the use of the water, for *Brycon cephalus* at a 0,1 L-1 larvae density, assuming an average depth of 1,2m and with no re-change, 10L per larva were required12. This calculation did not take into account loses due to evaporation and infiltration. In the results obtained with the lowest density (10 larvae L-1),

and with a daily re-change of 250 L day-1, the quantity of water required per larva was 2L in the same period. This clearly indicates that the recirculation system is environmentally beneficial in terms of water saving. Nevertheless, the sustainability of the quality of the water was only possible with energy consumption, which was produced by a hydroelectric plant, with a cost of U\$ 20,36 (178,5 KW) for 20 days operating the equipment.

Correlating the production obtained in the different treatments and their respective production costs, an economic evaluation of each one can be made. It was determined that the lowest density, even though it has the highest survival rate, is not the most profitable. This is mainly due to the low number of fish, which affects the indirect costs (24,17% higher than those of the highest density and 14,33% than those of the intermediate one). In the case of the highest density, the value of the supplies used in the larvae that die due to the high containment density, plus the low production volume, there is a direct costs increase of 24,17% compared to those of the lowest density and 9,84% compared to those of the intermediate one.

On the other hand, the intermediate density of 20 larvae per litter seems to be the best one, because it would generate enough profits to make the investment, which would be fully paid in 1,94 years keeping in mind that the values obtained were calculated according to the availability for reproducing the species (120 days a year) and not using the system in a permanent way.

Hatch and Tai¹⁴, after performing several simulations in fish farms to determine the factors that affect their profitability, discovered that the technology in low scale farms is profitable, thus indicating that the increase in the containment density of the animals and the sale Price significantly increase the economic indicators. Therefore, density is the most influential factor for the profitability. Therefore, not always a high containment density will mean a great return of the capital.

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