Producción artesanal del rotífero *Philodina* sp y de algas para la alimentación de post larvas de bocachico

Artisan production of the rotifer, *Philodina* sp. and algae as post-larval feed for the Colombian 'bocachico'.

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RESUMEN

El cultivo de algas mixtas se realizó en el Instituto de Piscícultura Tropical de la Corporación Autónoma Regional del Valle del Cauca (Buga 25 ^{o}C y 969 m.s.n.m.) utilizando fertilizantes inorgánicos en baldes plásticos, se produjeron en promedio 386 x 10^3 células/ml de cultivo. En el cultivo de *Philodina* en frascos de vidrio alimentado con algas y levadura, se obtuvieron 410 rotíferos/ml de cultivo. Se evaluaron tres tratamientos: rotíferos enriquecidos con aceite de pescado; rotíferos más algas *(Chlorella, Scenedesmus, Pediastrum, Spyrogira y Anabaena) y Artemia salina* + *Spirulina,* usando 100 post larvas de bocachico/acuario, alimentadas dos veces al día según biomasa sembrada. El mayor porcentaje de sobrevivencia, peso y talla se obtuvo con el alimento constituido por rotíferos enriquecidos con aceite de pescado (93 %,3.2mg, 6.86mm)), seguido de rotíferos + algas (80.67 %,2 mg, 6.1mm) y *Artemia*+ *Spirulina* (60.6 %,1.6mg, 6.06mm) respectivamente.

Palabras clave: *Prochilodus reticulatus magdalenae*; *Philodina*; Rotíferos; Levadura; Enriquecimiento nutricional.

ABSTRACT

In the Tropical Piscicultural Institute of the Regional Autonomic Corporation of the Cauca Valley (average temperature 25°C; 969 m.a.s.l.) research was carried out to produce and algal and rotifer cultures as living food for post-larval bocachico (*Prochilodus reticulatus magdalenae*). The culture of mixed algae was produced using inorganic fertilizers in plastic pails, obtaining an average of 386 x 10^3 cells/ml of culture. *Philodina* rotifers were cultured in glass bottles, fed on algae and yeast, an obtaining an average of 410 rotifers/ml. Three kinds of food were evaluated for survival rate, size and weight of post-larval bocachico: Rotifers enriched with fish oil; rotifers plus algae (*Chlorella, Scenedesmus, Pediastrum, Spyrogina* and *Anabaena)*; and only *Artemia salina* and *Spirulina*. A completely random design was used with three treatments and three repetitions, using 100 bocachico/aquarium post-larvae, fed twice a day according to sown biomass. The highest survival, weight and size was obtained with the feed of rotifers enriched with fish oil (93% 3.2 mg, 6.86 mm) respectively, followed by rotifers plus algae (80.67%, 2mg, 6.1mm), and then Artemia plus Spirulina (60.6%, 1.6mg, 6.06mm).

Key words: *Prochilodus reticulates magdalenae*; *Philodina*; rotifers; yeast; nutritional enrichment.

INTRODUCTION

The Colombian bocachico, *Prochilodus reticulatus magdalenae*, characterized by migratory habits, and widely distributed in the principal river basins of Colombia, has three critical periods for larval survival: at the time of first feeding; when the first curve of the digestive system develops at 15 days; and at 30 days when it starts to feed on pond detritus (De Fex, 1996).

The rotifer, *Philodina* (Class: Bdelloidea, Family: Philodinidae) filters organic particles in suspension (bacteria, detritus, algae, protozoa), and has a longevity of 48 days on average. The female lays 45 eggs, and the generation interval is four days (Ruppert y Barnes, 1996**;** Barnabé, 1996). The nutritional composition varies between 6.0% and 7.9% of crude protein, 1.4% and 3.7% of lipids, 0.16mg/g of calcium, and 1.1 to 1.5mg/g of phosphorus (Watanabe, Kitajima and Fujita, 1983). Rotifers can be fed with a large scale culture of *Chlorella,* obtaining enrichment in fatty acids of 12.7 to 18.8 % (Torrentera, 1989). The culture is simpler and less costly when using *Geotricum candidum*, a yeast that grows in lactic acid, milk whey, vinegar, milk, cheese and butter.

As it is necessary to establish cultivation techniques for obtaining a live food of an optimum size and a high nutrient content that favors growth and survival of post-larval bocachico, this study aimed to assess the production in the laboratory of algae as rotifer feed, together with *Geotrichum candidum*. In the study, the post-larval diet was enriched with fish oil and was compared with conventional feed (*Spirulina* and brine shrimp, *Artemia salina).*

MATERIALS AND METHODS

The study was carried out in the laboratory of the Tropical Pisciculture Institute of the Regional Autonomic Corporation of the Cauca Valley (CVC) (Buga, Valle del Cauca, 969 m.a.s.l.; 25°C) in two phases: the culture of algae and rotifers and the assay of post-larval feeding of the bocachico.

Assay 1. Culture of algae and rotifers

Amongst the plankton collected from ponds (using a net of 60 microns with collector recipient) the following algae were identified: *Chlorella, Scenedesmus, Pediastrum, Spyrogira* and *Anabaena*, and the rotifers: *Brachionus*, *Phylodina* and *Polyartra* (Nedham y Nedham, 1978). The algae were cultivated outdoors in plastic pots (11 l), and seeded initially with 300 ml of the sample using water from the River Guadalajara with a pH of 6.8.

The mixed algal cultures were organized in a completely randomized design with three treatments (T1: 1 g urea + 0.5 g of the fertilizer ‘agrimins’ per liter of water; T2: 2 g urea + 0.5 g ‘agrimins’ per liter of water; T3: 1 g of the fertilizer ‘”triple 15” (N, P, K) + 0.5 g ‘agrimins’ per liter of water), with three repetitions per treatment.

The number of cells/ml of water was assessed daily, using a random sampling of 100ml taken from each container. With the daily counts, the growth curves were plotted with the statistical package Curve Expert, and the Gaussian regression model with the correlation coefficients greater than 0.9, and with a minimum count of 138000 cells/ml. The growth curve was established using the model $y = a*\exp((-(-b-x)^2)/(2*c^2))$, where the derivative is the daily growth rate. This was then adjusted to a linear regression model, $y = a+ bx$, obtaining the average daily growth rate per treatment.

After repeated assays of rotifer isolation with a micropipette, each genus was separated in assay tubes of 5 cm^3 distilled water and fed with *G. candidum* for five days. As the greatest population was obtained of *Phylodina*, these were seeded at 30 individuals/ml in glass flasks (3 l) with distilled water at 23 ^{o}C and neutral pH.

In the culture of *Phylodina* two diets were used (0.003 g. of *G. candidum* enriched with fish oil for each 10 cm^3 of culture water; the same dose of *G. candidum* with artificially cultivated algae). The count was carried out using a McMaster chamber. Once the cultures were established, they were left four days for the population growth of the rotifer to average 400 individuals / ml, before refreshing them with the addition of distilled water and yeast.

Assay 2. post-larval feeding of bocachico

In the assay 900 3 day old larvae were used, with an average live initial weight of 6 X 10^{-4}g, and an average length of 4 mm. These were distributed in groups of 100 per aquarium. A completely randomized design was used, with three treatments. (T1: rotífers enriched with fish oil; T2:

Chlorella, Scenedesmus, Pediastrum, Spyrogira and *Anabaena*; T3: *Artemia salina* plus *Spirulina*) and three repetitions. Two daily feeds were provided (7:00 am and 12:00 pm), offered in the following manner: T1: 6 l of rotifer culture (415 rotifers/ml), T2: 6 l of rotifer culture plus 1000 ml per day of algal culture per aquarium, T3: 0.98 g of dry S*pirulina* in 600 ml of distilled water and 0.03 g of *Artemia salina* eclosed in acrylic cylinders at 25 ppt of salinity in a liter of water.

The duration of the assay was 10 days, and was started after the reabsorption of the viteline sack. Measures were taken of length (mm), weight (g), and survival (10% of the population extracted randomly per aquarium) (De Fex, 1996). The variables were submitted to an Analysis of Variance and Duncan tests of multiple ranges to separate the means.

RESULTS AND DISCUSSION

Mixed culture of algae

In the treatment 1, the maximum production of cells/ml was obtained on days 6; in treatments 2 and 3, on day 9. Nutrient scarcity began to show after 12 days (Table 1).

Table 1. Time of growth, and number of algae/ml per treatment

Culture days	Average T1	Average T2	Average T3
0	32000	32000	32000
1	37000	35667	32200
2	70667	84333	71333
3	118667	140667	114667
4	125667	180000	176333
5	135000	221333	245333
6	138333	230333	269667
7	116333	250333	288333
8	95667	275000	310667
9	90667	316000	389900
10	82667	315333	384000
11	52667	246333	327666
12	26333	192333	232666
13	19000	142333	146000
14	18667	111333	79333
15	18667	84000	63666
16	18333	52000	49666
17	18333	25333	25000

T1: 1 g. urea + 0.5 g. agrimins (minor elements) per liter of water.
T2: 2 g. urea + 0.5 g. agrimins per liter of water.
T3: 1 g. of Triple 15 (N, P, K) + 0.5 g. de agrimins per liter of water.

The regression model that best described growth was the Gaussian model, given the correlation coefficient (0.967, 0.982 and 0.979) (Table 2). As the greatest rate of growth, both observed and estimated, was in treatment T3 (Tables 3 y 4), this was selected for algal culture. No differences were found in the number of individuals (rotifers/ml) in the two diets used (Table 5).

Post-larval feeding of the bocachico

A statistically significant difference ($P < 0.01$) was seen in weight and length between treatments, but not in larval survival (Table 6). The best results of treatment 1 were attributed to the addition of fish oil, a source of polyunsaturated fatty acids (Omega 3 y Omega 6), and a limiting nutrients for the development and survival of larvae (Barnabé, 1996; Dantagnan *et al*., 1999). Also, the size of *Philodina* (90 µm) facilitates their capture by the larvae, which is not true of the brine shrimp of *A. salina* because of their size (2.4 mm) and the presence of appendages (De Fex, 1992).

Table 2. Gaussian model used for each treatment.

T1	T2	T3
y=a*exp((-(b-x)^2)/(2*c^2)) Coefficient: a = 134001.89 b = 5.8374261 c = 3.6441531 Correlation coefficient: 0.9674253 between expected and observed.	y=a*exp((-(b-x)^2)/(2*c^2)) Coefficient: a = 300021.33 b = 8.4643945 c = 3.9843125 Correlation coefficient: 0.9820830 between expected and observed	y=a*exp((-(b-x)^2)/(2*c^2)) Coefficient: a = 367815.82 b = 8.6331893 c = 3.5296032 Correlation coefficient: 0.9791118 between expected and observed

Table 3. Estimated daily growth rate of algae per treatment

Days	T1 Y' (t)	T2 Y' (t)	T3 Y' (t)
1	20225.18	24434.6	21742.22
2	22241.6	32799.55	33495.45
3	21144.39	40350.13	46539.64
4	16327.58	45045.58	57795.44
5	8229.935	44844.22	63151.83
6	-1638.85	38417.26	58857.7
7	-11149	25795.99	43323.3
8	-18298.5	8633.896	18395.84
9	-21898.3	-10110.5	-10771.6
10	-21875.3	-27005.1	-37439.1
11	-19097.5	-39170.5	-55808.8
12	-14882.9	-45079.4	-63069.5
13	-10473.9	-44825.9	-59974.4
14	-6703.09	-39822.6	-49872.3
15	-3918.96	-32136	-36944.1
16	-2099.71	-23780.9	-24633.3
17	-1033.28	-16233.3	-14878.7

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Table 4. Average daily rate of algal growth

Treatment	Mean values exponential phase cells/ml	Mean values descendent phase cells/ml.
T1	20.190	-10.700
T2	31.933	-38.365
T3	44.956	-43.444

Table 5. Number of rotifers obtained in each treatment

	Yeast + Fish oil	Yeast + algae
Number of individuals/ml seeded	30	30
Number of individuals/ml At day 4	415	410
$T^0 = 23^oC$ pH = 7.3.		

Table 6. Weight, length, and survival of post-larval bocachico on the 10^{th} day of treatment

Treatment*	Weight (gr)	Length (mm)	Survival (%)
T1	0.003[a]	6.86[a]	93[a]
T2	0.002[b]	6.13[b]	80.6[a]
T3	0.001[b]	6.06[b]	62.6[a]

Values with the same letter are not statistically different.
***T1:** Bocachico post larvae fed with rotifers enriched with fish oil
T2**:** Bocachico post larvae fed with rotifers and algae *(Chlorella, Scenedesmus, Pediastrum, Spyrogira and Anabaena*).
T3: Bocachico post larvae fed with Artemia salina and Spirulina.

Although the survival differences were not statistically significant, the distance of T1 from the conventional treatment (93% Vs 62.6%) showed the advantage of the live food. The result was also better than studies using *Brachionus plicatillis* (60%) (Verreth, 1999), rotifers and copepods between 150 – 230 µm (34 %), brine shrimp of *A. salina* (46 %), rotifers and brine shrimp *A. salina* (77 %) (Valderrama, 1992). These results were also better than the survival of post-larvae of bocachico (*Prochilodus nigricans*) fed with plankton produced in ground ponds (78 %), brine shrimp of *A. salina* (62 %), and plankton plus brine shrimp of *A. salina* (80 %) (Ascón, 1999), and that of *P. magdalenae* fed with wild zooplankton sized between 250 – 400 µm (49.9%) (Kerguelén, Sánchez and García, 2003).

CONCLUSIONS

The greatest population growth of the algal cultures (44.956 cells ml^{-1} day^{-1}) was obtained with 1 g of "triple 15" (N, P, K) + 0.5 gl^{-1} of agrimins.

In the post-larvae feeding stage the best result (93% survival, 0.0032g of weight and 6.86 mm of length) was obtained using rotifers enriched with fish oil.

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