

Effect of feeding frequency on Nile tilapia, *Oreochromis niloticus* (L.) fries performance during sex reversal in hapas

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ABSTRACT. Research aimed at verifying the necessary feeding frequency for the best performance of Nile tilapia, *Oreochromis niloticus* (Perciformes, Cichlidae) fries during sex reversal period in green waters. Approximately 1,600 seven-day-old fries, measuring 9.03mm and weighting 9.77mg, in a distribution of 80 fries/hapa, were fed on diet containing 43% of CP and 60mg of methyltestosterone/kg of diet at frequencies of 2, 3, 4, 5 and 6 feedings/day, divided equally over the light period, for 28 days. Parameters of growth, survival, uniformity, feed conversion and total biomass produced were measured. Growth was significantly ($p < 0.05$) reduced at the frequency of 2 times per day. Regression models were used to measure the effects of feeding frequency on total biomass, and on final average weight and length. Frequency of 4-to-5 feedings/day was the most adequate. Survival, variation coefficient, uniformity condition factor, feed conversion and cost variables were not affected by the feeding regimen. The results recommend the feeding of tilapia fries at least at 4 equally spaced times during the day during the sex reversal period in order to attain best performance.

Key words: tilapia, *Oreochromis niloticus*, fries, hapas, feeding frequency, sex reversal.

RESUMO. Efeito da frequência de alimentação no desempenho de larvas de tilápia do nilo, *Oreochromis niloticus* (L.), durante a reversão sexual em tanques rede.

Com o objetivo de testar a frequência da alimentação necessária para o melhor desempenho de larvas de tilápia do Nilo, *Oreochromis niloticus* (Perciformes, Cichlidae), durante o período de reversão sexual em águas verdes, 1.600 larvas, com idade aproximada de 7 dias, pesando 9,77 mg e medindo 9,03 mm, na quantidade de 80 larvas/tanque rede, essas larvas foram alimentadas com ração de 43% PB, contendo 60 mg de metil testosterona/kg de ração, nas frequências de 2, 3, 4, 5, e 6 alimentações/dia, divididas uniformemente, durante o período diurno, por 28 dias. Foram medidos os parâmetros de crescimento, sobrevivência, uniformidade, conversão alimentar e biomassa total produzida. O crescimento foi reduzido ($P < 0,05$) na frequência de 2 vezes/dia, e modelos de regressão foram utilizados para representar os efeitos da frequência de alimentação na biomassa total produzida, bem como para o peso e comprimento médios finais, que se mostraram mais adequados na frequência de 4 a 5 alimentações/dia. As variáveis sobrevivência, coeficiente de variação, uniformidade, fator de condição, conversão alimentar e custo da ração não foram afetados pelo regime alimentar. Conclui-se, portanto, que é necessário alimentar as larvas de tilápia durante a reversão sexual, pelo menos 4 vezes espaçadas durante o dia, para melhorar seu desempenho.

Palavras-chave: tilápia, *Oreochromis niloticus*, larvas, tanques-rede, frequência de alimentação, reversão sexual.

Tilapias have become well-known among fish farmers because of the advantages of rapid growth, rusticity (Torloni, 1984) and easy industrialization due to their lack of lateral musculature spines (Hiltsdorf, 1995). These qualities make tilapias the most adequate species for extensive and highly intensive cultivation, as the growing market has shown all over the world (Fitzsimmons, 1998).

The most difficult problem in tilapia cultivation is due to its precocious and constant reproduction, which causes overpopulation, competition and growth decrease. This may result in an excessive number of small and varying fish (Buddle, 1984; Mair and Little, 1991). Since male individuals of this species grow more than the females (Paiva *et al.*, 1988; Mair *et al.*, 1997), the cultivation of unisexual male population is of great interest. Techniques to

produce an all-male population has received closer attention because of its practical and good results. Sex reversal technique consists of feeding young fries with diets containing methyltestosterone (Wohlfarth, 1994; Macintosh and Little, 1995).

In the case of tilapia larviculture for all-male fry production, it is known that artificial diet with methyltestosterone must be fed from the very early days for a correct sex reversal (Guerrero, 1975; Hiott and Phelps, 1993). Technique is applicable to fish hatcheries or hapas in which natural diet (plankton) necessary to correct fry nutrition is available, without any damage to sex reversal (Chambers, 1984; Buddle, 1984; Popma and Green, 1990). The quality and the handling of the artificial diet used may vary.

Correct diet handling is absolutely necessary for fish growing performance without any sanitary risk. Besides metabolic-digestive alterations, excessive diet causes water quality deterioration and deficient diet results at a low growth rate and with great variations among individuals (Tabata *et al.*, 1988; Castagnolli, 1979). Thus the diet amount fed each time, or feeding frequency, may influence diet utilization. This is due to the fact that diet is directly applied to water and the non-uptaken portion will be dissolved and lixiviated. Feed conversion ratio increase and environmental pollution are the results. Since main aim is that fries uptake a high daily diet ratio to meet their nutrition requirement and thus ingest adequate hormonal amounts, and since high feeding frequency results in high daily diet intake ratio and small amounts of diet per feeding (Meer *et al.*, 1997), a higher frequency may be the most adequate.

Particularly in the sex reversal period of tilapia larviculture, experts' opinions about feeding frequency diverge from 2 times a day, as used by Guerrero's (1975) pioneer experiments, 2 to 4 times a day as recommended by Popma and Green (1990), three times a day as used by Alcazar (1988), four times a day as used by Phelps *et al.* (1995) and by Vera Cruz and Mair (1994), 6 to 8 times a day as reported by Carberry and Hanley (1997), up to the minimum of 8 times a day as recommended by Lim (1997).

With regard to the economic aspect, a higher feeding frequency would only be justified when absolutely necessary for a better performance. Needless to say, a higher feeding frequency would require higher costs with salary or with automatic feeders. Taking into account above biological and economic aspects of sex reverted tilapia fries production, the aim of this experiment was to verify the minimal feeding frequency required for an optimal performance in that period.

Material and methods

This experiment was carried out in the Piscicultura Piracema, municipality of Maringá PR Brazil, from March 9 to April 7, 1998. Twenty 1.5mm-mesh, 25x25x25cm plastic network cages with their edges outstanding 10cm above the water surface and with 12-liter capacity were used. Cages were fastened to the internal wall of a 80m² (10 x 8m) earth-bottom concrete tank supplied with artesian well water.

Nile tilapia *Oreochromis niloticus* (Perciformes cichlidae) fries from a 200 m² reproduction tank, containing 100 kg female and 50 kg male reproducers, were collected and separated according to technique developed by Popma and Green (1990). They were counted, weighted and stocked in randomly chosen and positioned cages at a rate of 80 fries/tank with 4 repetitions for each feeding frequency. A sample of forty fries was initially fixed in 5% formalith for further individual measuring and weighting on a 0.0001 g-precision analytical balance.

After being cage-stocked the fries were fed on a diet containing hormone. Diet was composed of two commercial initial rations mixed in equal portions and sifted through a 400 µ-mesh sieve. Its analysis is shown in Table 1.

Table 1. Composition of the diet used in the experiment

Nutrients	%
Dry matter	90.67
Crude protein	43.42
Crude fiber	2.66
Ethereal extract	8.08
Nonnitrogenized extract	25.76
Ash	10.75

The hormone 17α-methyltestosterone was incorporated into the diet, according to Guerrero (1975) technique, at a rate of 60 mg MT/kg diet and stocked at room temperature in a cool and shadowy place.

Diet rate used was 30% live weight/day, weekly readjusted and divided into feeding frequency according to treatments shown in Table 2. The fries were fed seven days a week over the experimental period of four weeks or 28 days; diet was moistened before being spread uniformly over the surface of the hapas.

Table 2. Feeding schedule and frequency

T	F/d	Feeding Timetable (hours)				
1	2	8				18
2	3	8	12			18
3	4	8	10	14		18
4	5	8	10	12	14	18
5	6	8	10	12	14	16

T = Treatments; F/d = Feeding/day

All fries were weekly collected, counted and weighted on a 0.1 g-precision electronic balance and thirty out of the total were measured individually on a 1.0 mm-precision ruler. At the end of the experimental period, fries were counted and weighted, and a sample of thirty individuals from each experimental unit was fixed in 5% formalith for a further individual weighting on a 0.0001 g-precision analytical balance.

At 8:00 a.m. and 6:00 p.m. the minimum and maximum temperatures and water pH were measured daily. Two nyctemeral measures of O₂ content, temperature and pH were taken at 6:00 a.m., 10:00 a.m., 2:00 p.m., 6:00 p.m., 10:00 p.m. and 2:00 a.m. on the 3rd and 18th day of the experiment. Daily measurement of water transparency was undertaken at 9:00 a.m., with a Secchi disk to verify primary production. Fish tank was not fertilized during the experimental period.

The formula $S_t = (80 - N_t) / 80 \times 100$, where S_t represents the weekly survival percentage, 80 the initial number of stocked fries and N_t the weekly number of fries, was used to calculate survival rate in percentage.

Length averages in millimeters and weight averages in milligrams were calculated for all individuals sampled in a week t , where t varied from 0 to 4 for each feeding frequency.

Variation coefficient (VC%), or rather, the percentage of mean samples, the formula $VC\% = S/X \times 100$ was used, in which X is the mean and S is the standard deviation for the variable weight or length.

Uniformity parameter for initial and final weights and for initial, weekly and final lengths was calculated by means of the formula $U\% = n_{30}/n \times 100$, where n_{30} is the number of individuals within the interval of 30% below and above the average and n is the total number of individuals sampled.

Total length and final weight data were submitted to polynomial regression analysis in which the freedom degrees of the variables were divided into linear, quadratic and cubic effects.

Statistical model for regression analysis was:

$$Y_{ij} = b_0 + b_1 f_i + b_2 (f_i)^2 + e_{ij}, \text{ in which}$$

Y_{ij} is the observation in the hapas j submitted to feeding frequency i ;

b_0 is the general constant;

b_1 is the linear regression coefficient of variable Y in relation to frequency i ;

b_2 is the quadratic regression coefficient of variable Y in relation to frequency i ;

f_i is the feeding frequency i ($i = 2, 3, 4, 5, 6$);

e_{ij} is the random error associated to each observation.

Duncan test ($p < 0.05$) was used to compare means and ($F < 0.05$) was considered for the variance analyses.

Results and discussion

Length and weight data from different feeding frequencies over the experimental period are shown in Tables 3 and 4 respectively. These variables, which presented a similar behavior, demonstrated growth inhibition ($p < 0.01$) for the twice-a-day frequency. This negative effect was detected from the second week up to the end of the experiment.

Table 3. Tilapia fry mean length (mm) at feeding frequencies

Frequency Times/day	Weeks					LG	GR/d
	Initial	1	2	3	Final		
2	9.025	12.79 ^a	14.03 ^b	16.96 ^b	21.39 ^b	12.36 ^b	0.44 ^b
3	9.025	13.60 ^a	15.83 ^a	19.37 ^a	23.23 ^a	14.20 ^a	0.51 ^a
4	9.025	13.29 ^a	15.68 ^a	19.41 ^a	23.00 ^a	13.97 ^a	0.49 ^a
5	9.025	13.18 ^a	15.64 ^a	19.36 ^a	23.41 ^a	14.38 ^a	0.51 ^a
6	9.025	13.98 ^a	15.58 ^a	19.13 ^a	23.23 ^a	14.20 ^a	0.51 ^a

Different letters in the same columns show differences ($p < 0.05$) by Duncan test; LG – Length gain = final length – initial length; GR/d – Length gain rate per day = LG/28

Table 4. Tilapia fry mean weight (mg) at feeding frequencies

Frequency Times/day	Weeks					LG	GR/d
	Initial (mg)	1	2	3	Final		
2	9.77	34.45 ^a	49.34 ^a	101.02 ^b	207.43 ^b	197.66 ^b	7.05 ^b
3	9.77	38.62 ^a	63.29 ^a	135.75 ^a	258.79 ^a	249.02 ^a	8.89 ^a
4	9.77	39.36 ^a	62.59 ^a	132.60 ^a	251.69 ^a	241.92 ^a	8.64 ^a
5	9.77	39.04 ^a	62.05 ^a	138.70 ^a	264.63 ^a	254.85 ^a	9.10 ^a
6	9.77	40.80 ^a	60.79 ^a	133.49 ^a	258.58 ^a	248.81 ^a	8.88 ^a

Different letters in the same columns show differences ($p < 0.05$) by Duncan test; WG – Weight gain = final weight – initial weight; WGR/d – Weight gain rate per day = WG/28

The effect of feeding frequency on fry's final length and weight means was quadratic ($p < 0.062$), as demonstrated in Figures 1 and 2 respectively.

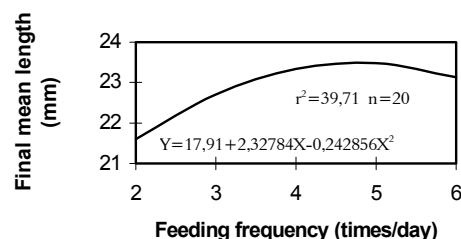


Figure 1. Nile tilapia fry's final mean length in different feeding frequencies

Derivation of the final mean lengths and weights in relation to feeding frequency in their respective

equations will produce the frequency in which these variables are maximal, at the point of $x = 4.79$ for both variables.

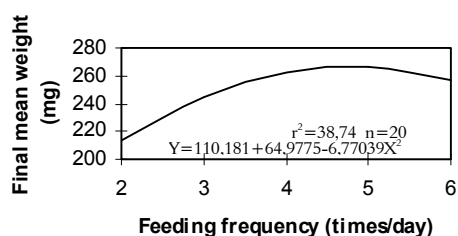


Figure 2. Nile tilapia fry's final mean weight in different feeding frequencies

The results of a preferential 4-5 times/day is in harmony with that 4 times/day frequency employed by Vera Cruz and Mair (1994) with tilapia fries in sex reversal phase. In Guerrero's (1975) pioneer experiment with tilapia sex reversal, a 2 times/day frequency was used which resulted in efficient sex reversal rates; but growth might be inhibited. In their investigation on Nile tilapia juveniles, Tung and Shiau (1991) found a better weight gain at a 6 times/day feeding frequency. However, these authors tested only 2 times/day and 6 times/day frequencies, and failed to mention intermediary frequencies. Jobling (1983) also reported that feeding frequency increase from 1 to 2 times/day for arctic char (*Salvelinus alpinus*) increased growth, although this author did not test higher frequencies. As for juvenile white sturgeon, Cui *et al.* (1997) reported that an automatic feeder proved to be the most efficient. Despite the existence of many other fish species and categories, different authors used tilapia fry 4 times/day feeding frequency in their investigations (Chambers, 1994; Popma and Green, 1990; Ridha and Lone, 1995; Phelps *et al.*, 1992, 1995; Abucay and Mair, 1997).

Variation coefficient (VC) and uniformity are antagonistic measures that reflect the same effect: the variation among individuals shown in Table 5.

Table 5. Other variables studied in terms of feeding frequency

Variables	Feeding frequencies (times/day)				
	2	3	4	5	6
Uniformity%	37.50	50.83	39.17	46.67	42.50
Survival%	86.56	88.75	79.69	91.25	94.06
VC%	57.20	51.82	55.74	54.46	59.51
AFC	2.61	2.47	3.22	2.45	2.44
Cost/1,000 (R\$)	0.60	0.78	0.94	0.74	0.75

Different letters in the same lines point out differences ($p < 0.05$) by Duncan test; VC% = variation coefficient; AFC = apparent food conversion; Cost/1,000 (R\$) = diet cost to produce 1,000 fries, in reais

In the present experiment, 2-to-6-times/day feeding frequency did not affect significantly ($p < 0.05$) the two variables. Feeding frequency effect on VC was studied by Jobling (1983) in arctic char (*Salvelinus alpinus*). Researcher reported a lower VC when feeding frequency increased from once a day to 2 times/day. A higher feeding frequency possibly decreases competition among individuals and results in a lower variation. Consequently a higher uniformity is reported. In the feeding frequencies used in our experiment, however, this effect was not expressive, probably due to plankton availability.

Survival rates were also not affected ($p > 0.05$) by the feeding regimen, as demonstrated in Table 5. Feeding frequency effect was not evaluated for arctic char (Jobling, 1983) and neither for the white sturgeon (Cui *et al.*, 1997). This variable seems to be more affected by other management factors or genetic problems (Macintosh and De Silva, 1984). Survival rate was relatively high during all the experiment (an average of 88.06%), which is probably due to natural food availability.

Apparent feeding conversion (AFC) results and diet cost to produce one thousand fries are also shown in Table 5. AFC data did not reveal significant differences in terms of feeding regimen. This fact is probably due to natural food availability, to high feeding rates (30% CP), and to the weekly adjustments during all the experiment. Data were particularly high at the four times/day frequency. This is due to mortality observed in some repetitions of this treatment which also increased the diet cost to produce one thousand fries. As might be expected it was higher in the treatments with a higher diet uptake and growth. In these frequencies, due to a higher growth and to the weekly adjustments, there occurred a higher diet uptake which resulted in a higher cost in terms of amount produced and food conversion and not of average final length.

The values of total biomass produced were represented by linear regression model, as shown in the Figure 3, where feeding frequency increase resulted in an increase of biomass produced.

Indicating primary production, the average transparency along the whole experimental period was 59.18 cm, with a maximum of 89 cm and a minimum of 32 cm. This fact indicated the presence of plankton, albeit not in excess.

Temperatures read at 8 a.m. and at 6 p.m., as well as the maximum and minimum temperatures, showed an average of 24.11, 25.67, 27.50, 25.67, respectively, having as minimum limit 17°C on the 11th and maximum limit 32°C on the first and 13th

day. They remained within the ideal limits for tropical species (Kubitza, 1998).

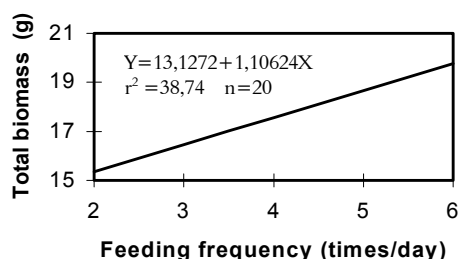


Figure 3. Biomass produced in terms of daily feeding frequency ($p < 0.05$)

Average pH verified at 8 a.m. during the experimental period was 7.34, within the limits of 6.64 and 8.19; average pH verified at 6 p.m. was 7.57, varying between 6.27 and 8.29. These limits are ideal for fish production, according to Kubitza (1998).

Dissolved oxygen rates measured over the nyctemeric variations were highly variable, but the lowest results were obtained very early in the morning. They reached minimum levels of 2.8 mg/L for all treatments. Even at minimum oxygen rates, however, there was no lack of oxygen symptoms which would be demonstrated by surface breathing. During the day, oxygen rate varied from 3.8 to 5.6 mg/L in all treatments. It may be preliminarily concluded that physical and chemical variables have not affected the results of this experiment. Taking into account the results of this experiment within its specific conditions it may be concluded that for a better performance of *Oreochromis niloticus* fries during sex reversal a feeding frequency of at least four times a day is necessary.

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