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GROWTH PERFORMANCE OF *Prochilodus lineatus* (= *Prochilodus scrofa*) LARVAE AND FRY – METHOD OF ANALYSIS BASED ON THE RELATIVE CONDITION FACTOR*

(Desempenho de crescimento de larvas e alevinos de *Prochilodus lineatus* (= *Prochilodus scrofa*) – Método de análise baseado no fator de condição relativo*)

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RESUMO

O objetivo deste trabalho foi avaliar o desempenho de crescimento de larvas e alevinos de *Prochilodus lineatus*, com base em um índice ponderal, o fator de condição relativo (Kn). Na primeira fase experimental, as larvas receberam as seguintes dietas vivas: rotíferos *Brachionus plicatilis* alimentados com levedura (LD1), rotíferos enriquecidos com fontes de ácidos graxos - *Chlorella homosphaera* (LD2), emulsão contendo óleo de fígado de bacalhau (LD3), emulsão contendo óleo de soja (LD4) - e plâncton selvagem (LD5). Após 30 dias, não foram observadas diferenças significativas entre as taxas de sobrevivência das larvas submetidas aos cinco tratamentos. Para análise do desenvolvimento das larvas, o Kn foi aplicado. As dietas LD1, LD4 e LD2 produziram os melhores resultados em termos de fator de condição relativo. No segundo período, com duração de 45 dias, os alevinos receberam uma dieta artificial básica (tratamentos AD2 e AD5), suplementada com levedura (AD1), óleo de fígado de bacalhau (AD3) e óleo de soja (AD4). As melhores taxas de sobrevivência foram observadas nos tratamentos AD2, AD4 e AD1, enquanto que os melhores valores de Kn foram encontrados nos tratamentos AD1, AD4 e AD2.
Palavras-chave: *Prochilodus lineatus*, larvas, alevinos, crescimento, fator de condição relativo

ABSTRACT

This work attempts to evaluate the growth performance of *Prochilodus lineatus* larvae and fry, fed on live and artificial diet, based on the relative condition factor (Kn). In the first experimental phase, the larvae were fed on live feed: rotifers *Brachionus plicatilis* fed solely with *Saccharomyces cerevisiae* baker's yeast (LD1), rotifers enriched with fatty acids sources - *Chlorella homosphaera* (LD2), cod-liver oil emulsion (LD3), soybean oil emulsion (LD4) - and wild plankton (LD5). After 30 days, there were no significant differences in the survival rates of the five treatments. To analyze the development of larvae, the Kn was applied. The diets LD1, LD4 and LD2 gave the best results. In the second period, the fry were fed with a basic artificial diet (AD2 and AD5 treatments), supplemented with yeast (AD1), cod-liver oil (AD3) and soybean oil (AD4). The highest survival rates occurred in AD2, AD4 and AD1, and the best Kn values, in AD1, AD4 and AD2.
Key words: *Prochilodus lineatus*, larvae, fry, growth, relative condition factor

Introduction

Fish larvae culture is considered one of the most difficult steps in the process of intensive fish production. Among the several factors responsible for the failure in this field, the feeding factor is the chief, since the quality and quantity of feed to be given to fish larvae is not yet unequivocally known. Some researchers (HORVÁTH, 1978; DABROWSKI, 1984; PERSON-LE RUYET, 1989; BENGTSON, 1991) believe that feeding with live microorganisms is still the most suitable procedure for the larvae of the majority of fish species, particularly in their first days of life.

Some studies on the feeding of indigenous fish larvae have been carried out in Brazil, particularly in the last decade. Among them are the studies of

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BASILE-MARTINS *et al.* (1987), YAMANAKA (1988) and DIAS; CASTAGNOLLI; CARNEIRO (1988), with *Piaractus mesopotamicus* (=*Colossoma mitrei*) larvae; FREGADOLLI (1990), with *P. mesopotamicus* and *Colossoma macropomum;* SIPAÚBA-TAVARES (1993) and SIPAÚBA-TAVARES and BACHION (1995), with larvae of *C. macropomum* and of the hybrid, *P. mesopotamicus X C. macropomum*, as well as the studies of CESTAROLLI and PORTELLA (1994), CESTAROLLI; PORTELLA; ROJAS (1997), SALLES (1998) and PORTELLA; VERANI; CESTAROLLI; (2000) and PORTELLA *et al.* (2000) with *P. scrofa* larvae.

One of the major obstacles to determine the effects of treatments on larvae performance is the heterogeneus growth that these animals present in this phase. According to OPUSZYNSKI *et al.* (1989), the variability of size is considered an undesirable event in larvae breeding. However little is known about the causes and effects of these significant variations. In their research on cyprinid larvae, those authors found weight variation coefficients ranging from 22 to 62% for common carp, 45 to 183% for large-headed carp, 23 to 86% for silver carp, and 27 to 101% for grass carp. DABROWSKI and POCZYCZYNSKI (1988) also found variation coefficients of up to 101.4% for common carp larvae.

VENKATESHVARAN; VENUGOPAL; REDDY (1993) used the relative condition factor to evaluate the performance of cyprinid fry ("catla", "rohu" and silver carp) treated with anabolic steroids. Similarly, this index was also used by LANDGE; VENKATESHVARAN; VENUGOPAL (1993) to evaluate the performance of *Clarias batrachus* bred in open-air tanks and in mixed breeding with other fish species.

In studies on *P. scrofa* larvae, PORTELLA; VERANI; CESTAROLLI (2000) found that a centralized value in an average does not always reflect the variation of the values observed, particularly those of weight. The authors suggest a method to evaluate growth results based on the classification of larvae into size categories.

The purpose of this work, therefore, is to continue that line of research using a weighty index – the relative condition factor (Kn) - defined by LECREN (1951), to evaluate the effects of the use of live and artificial feed enriched with essential fatty acids on the initial development of *P. lineatus*.

Material and Methods

The *Prochilodus lineatus* larviculture experiment was developed at the "Dr. Pedro de Azevedo" Freshwater Fish Biology Center in Pirassununga, state of São Paulo, Brazil. Two experimental phases were carried out: live diet enriched with essential fatty acids were tested for 30 days in the first phase, while artificial diets were used in the second, 45-day phase.

First experimental phase – live diets (LD)

Eight thousand *P. lineatus* larvae were equally distributed in twenty 10-liter tanks with a continuous flow system, at a population density of 40 larvae.L^{-1}. The experimental method employed was the Entirely Random one, consisting of five treatments, each one repeated four times.

The larvae were treated with the following diets: (LD1) rotifers *Brachionus plicatilis* fed with *Saccharomyces cerevisiae* yeast; (LD2) rotifers enriched with *Chlorella homosphaera* microalgae; (LD3) rotifers enriched with cod-liver oil emulsion, which has a high content of polyunsaturated fatty acids of the n3 series; (LD4) rotifers enriched with soybean oil emulsion, rich in linoleic and linolenic fatty acids; and (LD5) wild plankton organisms collected in manured ponds.

In the first four days from the start of exogenous feeding, the larvae were fed in daily proportions of 450 organisms.$larvae^{-1}$, divided into three meals, with the initial amount incremented by 450 organisms.$larvae^{-1}$ every five days thereafter.

Second experimental phase – artificial diets (AD)

At the end of the first phase, part of the resulting fry was distributed in twenty tanks with 40 litre of water with a continuous flow system, at a population density of 2 fry.L^{-1}, using the same experimental procedures described earlier. The artificial diets were formulated so as to maintain the principal sources of fatty acids employed. The composition and analysis of the artificial diets are described in PORTELLA; VERANI; CESTAROLLI (2000).

The fry of the previous LD1 treatment received the AD1 diet, rich in *S. cerevisiae* yeast; those of the LD3 and LD4 treatments received the AD3 and AD4 diets, formulated, respectively, with cod-liver and soybean oils. The fry resulting from the LD2 and LD5 treatments constituted the AD2 and AD5 treatments and received the same basic artificial diet.

During this phase, the feed was supplied *ad libitum*.

Analyzed parameters and statistics

At the end of both phases, the survival rates were

determined at each replication (S = 100 x number of survivors x initial number of larvae^{-1}) and biometries were performed on samples consisting of 10% of the individuals of each replication. Total wet weights (Wt) were determined employing an analytical scale and the total lengths (Lt), under a stereomicroscope equipped with a micrometric ocular.

BARTLETT test (1937) was applied to verify homoscedastic variance. The survival percentage rates were transformed into arcsine values for application of the Parametric Variance Analysis. The average of the results that showed significant differences were compared by the Tukey test using a 5% probability level.

The relative condition factor (Kn) was used to evaluate the growth results of the larvae and fry, employing the quotient: Kn = Wt.We^{-1}, where We was the weight theoretically expected.

The curves of the weight/length relation ($Wt = a.Lt^b$) were adjusted for each experimental phase, using the values of total weight (Wt) and total length (Lt) of the larvae and fry of all five treatments. The values of total weight theoretically expected (We) were calculated for each individual considering that $We = a.Lt^b$, based on the estimated "a" and "b" values.

The Kn values of all sampled larvae and fry were grouped into classes and the percentual frequencies of these classes were obtained for each experimental phase. Concurrently, the averages corresponding to the respective treatments were estimated for each experimental phase. The mean values were compared to the Kn= 1.0 standard using the Student "t" test ($\alpha = 0.05$). The Nonparametric Variance Analysis and the Kruskal-Wallis test, complemented by the Dunn test for grouping of series of similar Kn values, were applied to the individual Kn values for each treatment of each phase.

Results and Discussion

The adjusted weight/length curves ($Wt = a.Lt^b$) for each experimental phase were represented by the following equations: $Wt = 0.001348.Lt^{3.7155}$ for the first phase and $Wt = 0.009073.Lt^{3.0138}$ for the second one. After grouping the Kn values in classes, the percentual frequencies were obtained and are represented in Figures 1 and 2. The means corresponding to each treatment of both experimental phases were estimated using the Kn values (Figures 3 and 4).

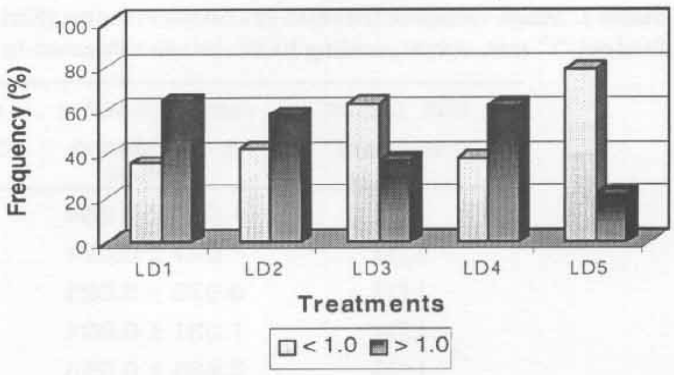

Figure 1. Frequency distribution of Kn values (1.0 > Kn > 1.0) of *P. lineatus* larvae in five treatments with live diets

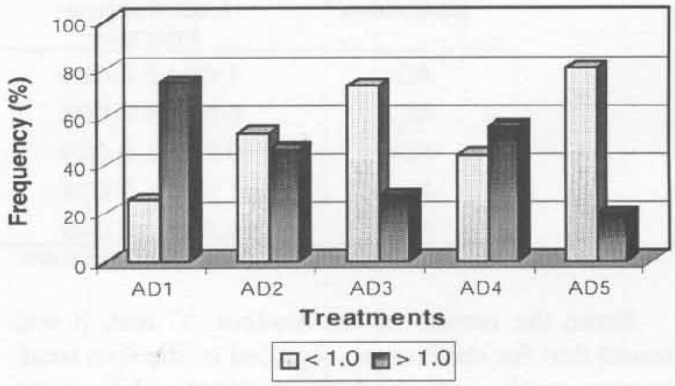

Figure 2. Frequency distribution of Kn values (1.0 > Kn > 1.0) of *P. lineatus* fry in five treatments with artificial diets

An analysis of the percentual frequency distribution of the Kn value grouping classes shows that in both live (Figure 1) and artificial diet (Figure 2) phases, the yeast treatments (LD1 and AD1, respectively) correspond to the highest frequencies in classes above value 1.0, while the treatments using cod-liver oil and wild plankton (LD3, LD5 and AD3, AD5, respectively), correspond to the highest frequencies in the classes with values below 1.0. The treatments using soybean oil and *C. homosphaera* (LD4, LD2 and AD4, AD2, respectively), correspond to frequency distributions that follow a pattern closer to normal distribution, i.e., centered around the average value of 1.0.

The mean values of Kn compared statistically to the Kn = 1.0 standard value using the Student "t" test (P = 0.05) are presented for *P. lineatus* larvae and fry on Tables 1 and 2, respectively.

Table 1. Mean values of the relative condition factor (Kn) with respective confidence intervals (CI) and the results of the Student "t" test, corresponding to the larvae subjected to five treatments with live diets

first phase treatment	mean Kn value ± confidence interval	number of larvae	estimated "t" Student	"t" test Ho: Kn=1.0
LD1	1.034 ± 0.034	75	2.306*	P<0.05
LD2	1.021 ± 0.021	69	1.723*	P<0.05
LD3	0.975 ± 0.025	76	1.754*	P<0.05
LD4	1.031 ± 0.031	58	2.088*	P<0.05
LD5	0.935 ± 0.065	28	2.622*	P<0.05

* significant

Table 2. Mean values of the relative condition factor (Kn) with respective confidence intervals (CI) and the results of the Student "t" test, corresponding to the fry subjected to five treatments with artificial diets

second phase treatment	mean Kn value ± confidence interval	number of fry	estimated "t" Student	"t" test Ho: Kn=1.0
AD1	1.064 ± 0.064	162	7.460*	P<0.05
AD2	0.994 ± 0.005	194	0.669^{NS}	P≥0.05
AD3	0.940 ± 0.059	100	6.636*	P<0.05
AD4	1.009 ± 0.009	184	1.158^{NS}	P≥0.05
AD5	0.950 ± 0.049	71	4.422*	P<0.05

(*) - significant (NS) - not significant

From the results of the Student "t" test, it was found that for the larvae subjected to the five treatments with enriched live diets, the mean values of yeast (LD1 - Kn=1.034), soybean oil (LD4 - Kn=1.031) and *C. homosphaera* (LD2 - Kn=1.021) treatments were significantly higher (P<0.05) than the standard Kn=1.0. Concurrently, the mean values of treatments with cod-liver oil (LD3 - Kn=0.997) and wild plankton (LD5 - Kn=0.935) were well below (P<0.05) the standard Kn=1.0 (Figure 3).

For fry fed with supplemented artificial diets, the mean value of the yeast (AD1- Kn=1.064) treatment was significantly higher (P<0.05) than the Kn=1.0 standard, while the treatments with soybean oil (AD4 - Kn=1.009) and *C. homosphaera* (AD2 - Kn=0.994) produced values that were very close (P≥ 0.05) to the Kn=1.0 standard. At the same time, the mean values of treatments using wild plankton (AD5 - Kn=0.950) and cod-liver oil (AD3 - Kn=0.940) were significantly lower (P<0.05) than the Kn=1.0 standard (Figure 4).

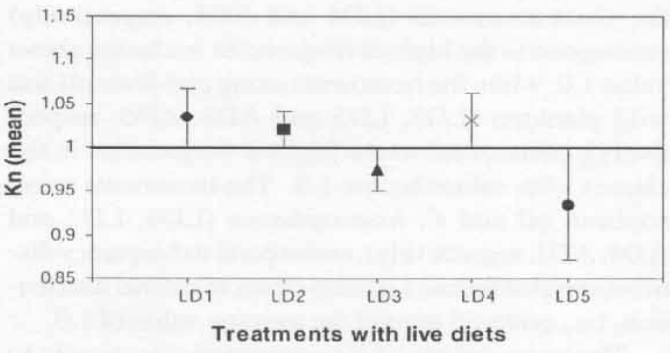

Figure 3. Mean values of relative condition factor (Kn + confidence interval) of the *P. lineatus* larvae subjected to different live diet treatments

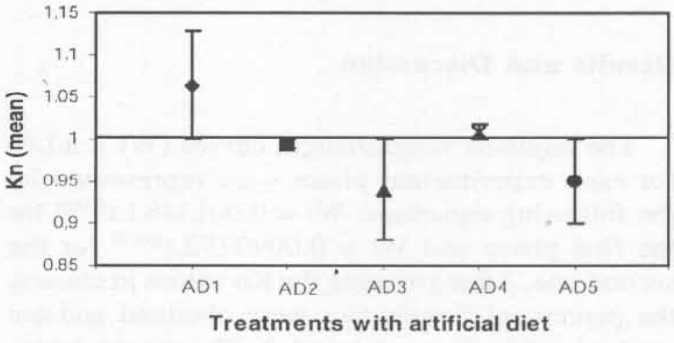

Figure 4. Mean values of relative condition factor (Kn + confidence interval) of the *P. lineatus* fry subjected to different artificial diet treatments

Applying the Nonparametric Analysis of Variance and the Kruskal-Wallis test on the individual Kn values for each treatment, a Ho rejection of P<0.05 was obtained for both the experimental phases. These results, complemented by the Dunn test to group the similar series of Kn values, are presented in Tables 3 and 4.

Table 3. First experimental phase – Mean ranking values (KW - Kruskal-Wallis test) and grouping results (Dunn's test) for the series of Kn data of *P. lineatus* larvae

treatment	mean ranking	N	KW * (P<0.05)	Dunn test
LD1	171.3	75	2198.0*	a
LD2	164.7	69		ab
LD3	130.5	76		bc
LD4	172.8	58		ab
LD5	100.5	28		c

(*) - significant

Table 4. Second experimental phase – Mean ranking values (KW - Kruskal-Wallis test) and grouping results (Dunn's test) for the series of Kn data of *P. lineatus* fry

treatment	mean ranking	N	KW (P<0.05)	Dunn test
AD1	465.6	162	-64696.0*	a
AD2	343.9	194		b
AD3	242.2	100		c
AD4	372.5	184		b
AD5	256.6	71		c

(*) - significant

Table 5. Mean survival rates of *P. lineatus* larvae and fry fed with live and artificial diets

first phase treatment	survival ($arcsine\ \sqrt{x/100}$)	second phase treatment	survival ($arcsine\ \sqrt{x/100}$)
LD1	43.8 [a1/] (48.1%)[2/]	AD1	47.8 [a] (54.7%)
LD2	41.6 [a] (44.4%)	AD2	52.7 [a] (63.1%)
LD3	46.5 [a] (52.6%)	AD3	34.5 [b] (32.2%)
LD4	37.0 [a] (36.1%)	AD4	50.5 [a] (59.4%)
LD5	30.1 [a] (25.4%)	AD5	39.9 [ab] (41.2%)

[1/] Values in rows with different superscripts are statistically different according to Tukey's test (p<0.05)
[2/] Values between parentheses are percentage of survival

The results of *P. lineatus* larvae and fry survival rates are given in Table 5. ANOVA results do not indicate statistically significant differences (P>0.05) among the survival rates during the first experimental phase. However, considering the relative condition factor results (Table 3), larvae of treatments LD1, LD4 and LD2 presented best performance than those of LD5 treatment, mainly if it is considered that the former treatments showed average Kn values highest than the centralized 1.0 value (Table 1 and Figure 3).

Thus, still in regard to the results of the second experimental phase, the fry fed for over 45 days with

a yeast-supplemented diet (AD1) presented the best performance of relative condition factor (Table 4), while the fry that received a cod-liver oil enriched diet (AD3) showed the worst survival performance (Table 5) and the lowest relative condition factor (Table 4), followed by the fry of the AD5 (basic diet) treatment. Intermediate development results were observed in the fry treated with soybean oil (AD4) and AD2 (basic diet).

The results obtained from the above experiments, therefore, lead us to propose an alternative criterion, based on the relative condition factor, to evaluate the performance of larvae and fry, with the purpose of optimizing experimental larvae treatments and, consequently, the fish seed production.

One of the practical advantages of applying the relative condition factor method is that it allows for statistical comparisons of the estimated Kn values against the centralized 1.0 value, regardless of species, length of the individuals (ANDERSON and GUTREUTER, 1983) or age. This does not hold true for the K factor which, in its formulation, is totally dependent on the length and weight of the animals ($K = W_t/L_t^b$).

Similar results were found by VERANI *et al.* (1997) who, considering the results of induced reproduction experiments on five rheophilic species of the São Francisco river basin, *Brycon lundii, Salminus brasiliensis*, *Leporinus elongatus*, *Prochilodus affinis* and *Prochilodus marggravii*, proposed a criterion to evaluate females suitable for induced reproduction based on the relative condition factor, with the intention of maximizing the success rates of this process.

ZAVAGLIA-PASCHOALINO *et al.* (1997), based on fish breeding experiments with *Oreochromis niloticus*, in which the relative condition factor methodology was applied to analyze the performance of the species in confined environments, and where post-winter sexing acts was a variation factor among the different breeding treatments, also propose a criterion to evaluate the performance of the fish, founded on the relative condition factor, aiming to optimize experimental treatments in fish culture.

Considering the results of *P. lineatus* larvae and fry performance, one can conclude that the species, in its initial phases of development, has low requirements for highly unsaturated fatty acids. According to PORTELLA *et al.* (2000), vegetable oils are recommended to fish species that require up to 18 carbon chain fatty acids, of both n6 and n3 groups. These authors found that body composition results of larvae and fingerlings of all treatments showed that linoleic acid was always preserved. This seems to indicate that 18:2n6 is essential for *P. lineatus*, at least during those developmental stages. Therefore, it is suggested that the use of soybean oil in these animals' diet, particularly in the larvae and fry phases, could be beneficial, since this oil is rich in linoleic acid.

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