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The effects of stocking density, prey concentration and feeding on *Rhinelepis aspera* (Spix & Agassiz, 1829) (Pisces: Loricariidae) larviculture

José Cláudio Epaminondas dos Santos[1*], Marcelo Mattos Pedreira[2] and Ronald Kennedy Luz[3]

*[1]Centro Integrado de Recursos Pesqueiros e Aquicultura de Três Marias, Av. Geraldo Rodrigues dos Santos, s/n, Satélite, Cx. Postal 11, 39205-000, Três Marias, Minas Gerais, Brazil. [2]Laboratório de Aquicultura e Ecologia Aquática, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, Minas Gerais, Brazil. [3]Laboratório de Aquacultura, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil. *Author for correspondence. Email: jose.claudio@codevasf.gov.br*

ABSTRACT. The aim of this study was to evaluate three stocking densities (20, 40 and 60 larvae L^{-1}), and two daily prey concentrations (400 and 700 *Artemia* nauplii larvae^{-1}) during the first seven days of active feeding (first phase). In the second phase was evaluate the effect of the different feeding regimes: fasting, dry diet (55% crude protein), *Artemia* sp. and mixed feeding (*Artemia* sp. plus dry diet), after 17 days of active feeding on *Rhinelepis aspera* larviculture. In the first phase, growth was affected only by prey concentration. In the second phase, the dry diet induced higher growth rates than fasting, but lower growth rates than the other treatments. The acceptance of the dry diet was evidenced by an increase in the growth rate from 4.6% day^{-1} in the first five days to 10.7% day^{-1} in the following five days of feeding. Survival was similar among dry diet, mixed feeding and *Artemia* nauplii treatments. *R. aspera* larvae can be reared during the first seven days of active feeding at a density of 60 larvae L^{-1}, after which they can be fed with a commercial dry diet.

Keywords: "Cascudo preto", commercial diet, feeding, ornamental fish.

Efeitos da densidade de estocagem, concentração de presas e alimentação na larvicultura de *Rhinelepis aspera* (Spix & Agassiz, 1829) (Pisces: Loricariidae)

RESUMO. O objetivo deste trabalho foi avaliar três diferentes densidades de estocagem (20, 40 e 60 larvas L^{-1}), e duas concentrações diárias de presa (400 e 700 náuplios de *Artemia* larva^{-1}) durante os primeiros sete dias de alimentação (primeira fase). Na segunda fase foi avaliado o efeito de diferentes regimes alimentares: jejum, dieta comercial (55% proteína bruta), *Artemia* sp., e alimentação conjunta (*Artemia* sp. mais dieta comercial), após 17 dias de alimentação, na larvicultura de *Rhinelepis aspera*. Na primeira fase, o crescimento foi afetado somente pela concentração de presas. Na segunda fase, o uso da dieta comercial proporcionou melhor crescimento comparado ao jejum e menor comparado aos demais tratamentos. A aceitação da dieta comercial ficou evidente pela taxa de crescimento específica que aumentou de 4,6% dia^{-1} nos primeiros cinco dias para 10,7% dia^{-1} nos cinco dias seguintes de alimentação. A sobrevivência foi similar para o uso de dieta comercial, náuplios de *Artemia* e alimentação conjunta. Larvas de *R. aspera* podem ser cultivadas durante os primeiros sete dias de alimentação em densidades de 60 larvas L^{-1}, e após este período inicial podem ser alimentadas com dieta comercial.

Palavras-chave: Cascudo Preto, ração comercial, alimentação, peixe ornamental.

Introduction

Intensive Neotropical fish larviculture has grown significantly in the last few years, bringing about great development in the knowledge of optimal rearing conditions for different species. During intensive larviculture, management of the stocking density is very important as it affects larval survival (CAMPAGNOLO; NUÑER, 2006; CHAKRABORTY; MIRZA, 2007; LUZ; SANTOS, 2008; LUZ; ZANIBONI FILHO, 2002) and growth (BASKERVILLE-BRIDGES; KLING, 2000; BOLASINA et al., 2006; IMOROU TOKO et al., 2008; LUZ; SANTOS, 2008) of different species. Optimal stocking densities for Neotropical freshwater larviculture are not defined for most species. Generally, densities between 15 and 30 larvae L^{-1} are used (ZANIBONI FILHO, 2000), although, densities up to 90 larvae L^{-1} (LUZ; PORTELLA, 2005), and 60 larvae L^{-1} (LUZ; SANTOS, 2008) have been recommended for *Hoplias lacerdae* and *Lophiosilurus alexandri* larvae, respectively.

The prey concentrations used during larviculture can also affect growth (RABE; BROWN, 2000;

SANTOS; LUZ, 2009), survival (DOU et al., 2003; GIMÉNEZ; ESTÉVEZ, 2008; HERNÁNDEZ-CRUZ et al., 1999), cannibalism (LUZ; ZANIBONI FILHO, 2001) and larval feeding behavior (GEORGALAS et al., 2007; PUVANENDRAN; BROWN, 1999). Furthermore, the provision of appropriate amounts of food avoids waste.

Despite the importance of live food during in larviculture, there is a need for research that can help formulate suitable dry diets for larvae and juveniles with the objective to diminish live food dependency (LEE, 2001). Weaning from live food is possible a few days or weeks after the initiation of exogenous feeding and can be accomplished by simultaneously feeding the target species with live food and a formulated diet (co-feeding) or without this co-feeding period (BASKERVILLE-BRIDGES; KLING, 2000; CALLAN et al., 2003; HART; PURSER, 1996; IMOROU TOKO et al., 2008; PETKAM; MOODIE, 2001).

The cascudo preto *Rhinelepis aspera* (Pisces:Loricariidae) shows detritivore feeding behavior and occurs in the São Francisco river and Paraná river, Brazil (AGOSTINHO et al., 1995; SATO et al., 1998). This fish is characterized by good tasting meat, few bones and practically no fat. It is listed among the 10 species of great importance to fishing in the São Francisco river basin, Minas Gerais State, Brazil. In the Paraná river, its population has been Declining due to dam construction (AGOSTINHO; GOMES, 2002). However, little is known about effective larviculture for this species. Improved information could be important in the reservoir stocking program and in the production of juveniles for the ornamental aquarium industry.

The aim of this study was to evaluate the effects of different prey concentrations and stocking densities during the first seven days of active feeding and, later, the effects of different feeding regimes, on *Rhinelepis aspera* larviculture.

Material and methods

The experiment was conducted at the Integrated Center of Fisheries and Aquaculture Resources of Três Marias-Codevasf, Minas Gerais State, Brazil, and was divided into two experimental phases.

First experimental phase

Larvae of *Rhinelepis aspera* were obtained by induced spawning. Mature females and males received a hormonal induction treatment by intramuscular injection with carp pituitary extract (WOYNAROVICH; HORVÁTH, 1983). Oocytes and sperm were obtained through a single extrusion using abdominal pressure, and fertilized eggs were kept in upward flow incubators (20-L).

At five days post-hatching, *R. aspera* larvae (total length of 6.2 ± 0.3 mm and wet weight 3.2 ± 0.1 mg, onset of exogenous feeding) were stocked in 18 circular aquaria (2 L volume and totally white), where the dissolved oxygen concentration was maintained higher than 5.0 mg L^{-1} and the water temperature at 26.5 ± 0.5°C and 27.3 ± 1.1°C at 9 and 17h, respectively. The photoperiod was 10h light and 14h dark, and the light level at the water surface was 150 lx.

During the first seven days of active feeding, three stocking densities (D) were used: D20 = 20 larvae L^{-1}; D40 = 40 larvae L^{-1}; and D60 = 60 larvae L^{-1}. For each density, two daily prey concentrations (P) were applied: P400 = 400 *Artemia* nauplii $larvae^{-1}$ and P700 = 700 *Artemia* nauplii $larvae^{-1}$. These prey concentrations were applied during the first five days of active feeding. During the 6^{th} and 7^{th} days of active feeding, these concentrations were halved in 50% of initial prey concentration. Larvae were fed three times per day at 9, 13 and 17h. The experimental design was a 3 x 2 factorial design with three stocking densities, two prey concentrations, and three replicates of each.

Each day the aquaria were siphoned to remove waste before the first and last feedings, resulting in about 60% of the total water volume being renewed.

Second experimental phase

After seven days of active feeding (12 days post-hatching), larvae under the P700D60 treatment had a total length of 15.4 ± 1.3 mm and wet weight of 46.3 ± 12.4 mg. They were then stocked in 20 circular aquaria (3 L volume), similar to those used in the first phase. The stocking density was 10 larvae L^{-1} and the water temperature was maintained at 25.5 ± 0.5°C and 26.3 ± 0.6°C at 9 and 17h, respectively.

The following four feeding regimes were applied: (1) fasting; (2) commercial dry diet (diameter < 0.5 mm, 55% crude protein, 10% moisture, 4% ether extract, 6% fiber matter, 18% mineral material, 5% calcium, and 1.5% phosphorus); (3) *Artemia* sp.; and (4) mixed feeding (*Artemia* sp. plus commercial dry diet). The experimental design was completely randomized with four treatments of five replicates each.

For the *Artemia* sp. treatment, larvae were fed three times per day at 9, 13 and 17h, with daily quantities of 1,050; 1,400 and 1,750 *Artemia* nauplii $larvae^{-1}$ from the 8^{th} to 10^{th}, 11^{th} to 15^{th} and 16^{th} to 17^{th} days of active feeding, respectively. For the

mixed feeding treatment, the *Artemia* nauplii concentration was half of that used in the *Artemia* treatment. The commercial dry diet was offered ad *libitum*.

Each day the aquaria were siphoned for cleaning before the first feeding, resulting in about 50% of the total water volume being renewed.

Evaluated parameters

The ammonium ion concentration was analyzed on the 6th day of active feeding in the first experimental phase, and on the 12th and 17th days of active feeding in the second phase, following the Koroleff (1976) method.

At the end of the first phase, survival was evaluated and samples of 15 animals of each replicate were weighed to the nearest 0.0001 g and total length was measured with digital calipers (Starrett).

In the second phase, the same measurements were taken on samples of five animals after 12 days of active feeding and on samples of 15 animals at the end of the study from each replicate, when survival was determined.

The larval specific growth rate (SGR) was determined using the following relationship: SGR = 100 x (*Ln* Wtf – *Ln* Wti) Δt^{-1}, where Δt is the time interval (in days) between Wti (initial weight) and Wtf (final weight) measurements.

Statistical analysis

The survival and specific growth rate data from the two experimental phases were arcsin transformed for statistical analysis. For the first phase, data were compared by parametric factorial ANOVA and group means were compared using Tukey's multiple range test, at 5% probability level.

The second phase data were compared using one-way ANOVA and group means were compared using Tukey's test, at 5% probability level. SigmaStat 3.5 was used for all statistical analyses.

Results and discussion

None of the analyzed parameters were affected by stocking density × prey concentration ($p > 0.05$) interactions (Table 1). The stocking density did not affect growth parameters ($p > 0.05$), but *R. aspera* weight, length and SGR were affected by prey concentrations ($p < 0.01$). The best results came from the P700 group. Survival was not significantly affected ($p > 0.05$) by any treatment. Ammonium ion concentration was affected only by stocking density ($p < 0.01$), with lower concentrations for D20 and higher levels for D40 and D60, which had similar levels between them.

In the second phase, survival was not significantly different between *Artemia* (80.6 ± 6.4%), commercial dry diet (76.0 ± 5.5%) and mixed feeding (74.6 ± 4.5%) treatments. However, the fasting had significantly lower survival (58.3 ± 8.4%; $p < 0.05$).

The fasting treatment showed the lowest growth rates (length and weight; Table 2). The growth rate for the commercial dry diet treatment was lower when compared with the other treatments. The mixed feeding treatment *R. aspera* larvae weighed less ($p < 0.05$) than the *Artemia* treatment larvae after twelve days of active feeding; however, no differences in length were observed ($p > 0.05$). After 17 days of active feeding, growth did not significantly differ ($p > 0.05$) between mixed feeding and *Artemia* treatments.

R. aspera larvae under the fasting treatment had the lowest SGR values ($p < 0.05$) after 12 days of active feeding, including negative SGRs, with only a slight recovery during the study (Table 2). *R. aspera* larvae under the commercial dry diet and mixed feeding treatments had lower SGR values ($p < 0.05$) compared with those in the *Artemia* treatment after 12 days of active feeding. However, SGRs were similar for animals under the commercial dry diet, *Artemia*, and mixed feeding treatments in the subsequent period of evaluation (among 13 to 17 days of active feeding).

Table 1. F values and means values (± standard deviation) of weight, total length (TL), specific growth rate (SGR), and survival of *Rhinelepis aspera* larviculture, and ammonium ion concentrations during the first experimental phase.

Statistical	F values				
	Weight (mg)	TL (mm)	SGR (% day^{-1})	Survival (%)	Ammonium ion (µg L^{-1})
Density (D)	2.42ns	2.58ns	2.65ns	0.14ns	31.02**
Artemia nauplii concentrations (P)	15.42**	15.95**	13.81**	0.03ns	3.34ns
Interaction D x P	0.66ns	0.69ns	0.93ns	1.08ns	0.24ns
Treatments	Means for D				
D20	30.4 ±15.9	13.4 ± 2.2	30.4 ± 8.0	75.5 ± 16.1	1410.8 ± 263.1b
D40	4 1.9 ± 23.4	14.5 ± 2.5	34.4 ± 9.8	81.0 ± 18.4	2076.3 ± 194.5a
D60	48.9 ± 12.3	15.6 ± 1.5	38.6 ± 3.8	81.6 ± 9.2	2292.4 ± 46.1a
	Means for P				
P400	25.4 ± 11.9b	12.7 ± 1.9b	28.1 ± 7.3b	78.1 ± 14.3	1752.8 ± 471.3
P700	50.9 ± 14.8a	15.8 ± 1.3a	38.9 ± 4.7a	80.3 ± 16.6	2004.3 ± 414.7

Means followed by the different letters in vertical comparisons differ significantly by Tukey's test. *($p < 0.05$); ** ($p < 0.01$); ns (Not significant).

The ammonium ion concentration was lower ($p < 0.05$) in the fasting treatment during the study (Table 2). After 12 days of active feeding, ammonium ion concentrations for the commercial dry diet treatment were lower ($p < 0.05$) compared to the live food and mixed feeding treatments, which were similar to one another ($p > 0.05$). At the end of the second phase, the commercial diet, *Artemia* and mixed feeding treatments had similar ammonium ion concentrations ($p > 0.05$).

Artemia nauplii can be used as first food for *R. aspera* larvae. This result corroborates other studies of fish larvae for several Neotropical freshwater species with different feeding behaviors (JOMORI et al., 2003; LUZ; ZANIBONI FILHO, 2001; LUZ; PORTELLA, 2002; SANTOS; LUZ, 2009).

For *R. aspera* larviculture, prey concentration management becomes very important during the first seven days of active feeding, where faster growth is related to higher prey concentrations. In other species exhibiting different feeding behaviors, such as *Gadus morhua* (PUVANENDRAN; BROWN, 1999), *Sebastes* spp. (LAUREL et al., 2001), *Paralichthys olivaceus* (DOU et al., 2003), *Dentex dentex* (GIMÉNEZ; ESTÉVEZ, 2008), *Prochilodus costatus* and *Lophiosilurus alexandri* (SANTOS; LUZ, 2009), differences in prey concentration also affected growth. The slower rate of growth at low prey concentrations can be explained by higher energy costs spent in food capture for larvae that swim in the water column (PUVANENDRAN; BROWN, 1999). However, *R. aspera* larvae exhibited movement only during feeding times and remained at rest most of the time. This suggests that these animals spend very little energy on swimming and food capture. This fact was also observed in *L. alexandri* larviculture (SANTOS; LUZ, 2009). These observations reveal that the minor prey concentration used in *R. aspera* larviculture was low for this species. Additional studies that evaluate higher prey concentrations than the present work, plus other factors like higher stocking density, feeding frequency, or different water temperatures, can provide more information on *R. aspera* feeding.

Interestingly, we found that stocking density did not affect growth parameters. This result is similar to findings for *G. morhua* (BASKERVILLE-BRIDGES; KLING, 2000), *Dicentrarchus labrax* (HATZIATHANASIOU et al., 2002), *Hoplias lacerdae* (LUZ; PORTELLA, 2005), and *Pseudoplatystoma corruscans* (CAMPAGNOLO; NUÑER, 2006) larviculture. For *Prochilodus scrofa*, a detritivore species reared in densities of 0.5, 0.75 and 1 larvae L^{-1}, the highest weight gain was registered at the lowest density after 45 days, but with similar values after 68 days (KOBERSTEIN; DURIGAN, 2001), thus revealing the effect of density on animal development. Despite the higher ammonium ion concentrations for the D40 and D60 treatments, this had no apparent influence on animal growth and survival. An increase in the concentration of ammonia with increased stocking density has been observed for *H. lacerdae* (LUZ; PORTELLA, 2005), *G. morhua* (BASKERVILLE-BRIDGES; KLING, 2000), *P. corruscans* (CAMPAGNOLO; NUÑER, 2006) and *L. alexandri* larviculture (LUZ; SANTOS, 2008), indicating the need for better water quality care with increasing stocking densities.

In the second phase, survival was lower ($p < 0.05$) for larvae in the fasting treatment. However, the different feeding regimes showed that survival was higher (> 74%) and similar between them. These results are positive and suggest that the commercial dry diet provided a good diet for this species.

The commercial dry diet induced better growth compared to fasting and lowers to the other treatments. However, its use during the first days of feeding presents a problem for most species, which show the lowest growth attributed to the diet quality and digestibility (HUNG et al., 1999) and to the larvae not accepting the diet (LEE; OSTROWSKI, 2001). However, other detritivore freshwater species like *Loricariichthys platymetopon* (HAYASHI et al., 2002) can be fed with the dry diet as they exhibit exogenous feeding even during the first days of feeding.

Table 2. Means (± standard deviation) of weight, total length (TL), and specific growth rate (SGR) of *Rhinelepis aspera* and ammonium ion concentrations during the second experimental phase.

	Data after 12 days of active feeding			
Treatments	Weight (mg)	TL (mm)	SGR (% day^{-1})	Ammonium ion (μg L^{-1})
Fasting	39.5 ± 2.2d	15.7 ± 0.5c	-3.1 ± 1.1d	930.4 ± 44.2c
Commercial dry diet	58.0 ± 3.1c	17.0 ± 0.3b	4.6 ± 1.0c	2117.8 ± 29.4b
Artemia nauplii plus commercial dry diet	73.6 ± 11.7b	17.8 ± 0.6ab	9.2 ± 2.9b	2231.7 ± 48.0a
Artemia nauplii	91.1 ± 11.9a	18.8 ± 0.7a	13.5 ± 2.5a	2277.2 ± 11.6a
	Data after 17 days of active feeding			
Fasting	39.9 ± 5.6c	16.3 ± 0.2c	-0.3 ± 2.9c	768.8 ± 69.9b
Commercial dry diet	99.0 ± 5.1b	19.5 ± 0.3b	10.7 ± 1.6b	2178.2 ± 99.8a
Artemia nauplii plus commercial dry diet	179.8 ± 13.7a	22.9 ± 0.5a	17.9 ± 3.0a	2316.2 ± 11.1a
Artemia nauplii	179.1 ± 19.9a	23.5 ± 0.5a	13.5 ± 2.9ab	2290.7 ± 40.1a

Means followed by the same letters in horizontal comparisons did not differ by Tukey's test ($p < 0.05$).

Mixed feeding revealed a similar growth response to the *Artemia* feeding treatment by the end of the study. Mixed feeding also induced a higher growth response compared to the exclusive commercial dry diet and a better and/or similar growth response compared to the use of live food (CAÑAVATE; FERNÁNDEZ-DÍAZ, 1999; FERMÍN; BOLIVAR, 1991; HAYASHI et al., 2002; KESTEMONT; STALMANS, 1992).

The SGR showed the worst response to the fasting treatment, with negative values in the first five days of the second phase and a slight recovery between the 12th and 17th days of active feeding. The slight recovery occurred because this species can use waste for growth. When the exclusively commercial dry diet was used, the pattern observed in the SGR showed that the animals pass through an adaptive process. During the first five days of feeding, a lower SGR (4.6%) was observed for *R. aspera* larvae in the commercial dry diet treatment compared with larvae in the *Artemia* treatment (13.5%). However, in the following period the SGR for larvae in the dry diet treatment rose to 10.7%, while larvae in the *Artemia* treatment maintained their SGR at 13.5%. This result indicates that nutritional studies can reveal solutions to improve diet intake and larval performance. *R. aspera* larvae in the mixed feeding treatment also showed a lower SGR in the first evaluation period (9.2%) and similar values to larvae in the *Artemia* treatment in the following period (17.9%). This suggests that, in addition to the half quantity of *Artemia* nauplii provided, the commercial dry diet supplied the animals' requirements and compensated for the reduction of live food.

The high ammonium ion concentrations found in response to the different feeding regimes were verified, as in the first phase, and were found not to affect *R. aspera* growth and survival.

According to these findings, our results showed that the use of live food for *R. aspera* larvae can be reduced to seven days of active feeding, as for *Rhamdia quelen* (BEHR et al., 2000), *Clarias macrochepalus* (PETKAM; MOODIE, 2001), *Pangasius bocourti* (HUNG et al., 2002) and *Heterobranchus longifilis* larvae (IMOROU TOKO et al., 2008), where a dry diet can be offered after a period of 3-7 days, 5-15 days, 4-6 days and 5-14 days of initial active feeding for the four species, respectively.

Conclusion

The cascudo preto *R. aspera* proved to be a potential intensive larviculture species using *Artemia* as food and with stocking densities up to 60 larvae L^{-1} during the first seven days of active feeding. After this initial period, *R. aspera* larvae can be fed exclusively on a commercial dry diet.

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