



Optimal feeding frequency for *Heros severus* (Heckel, 1840), an Amazon ornamental fish

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Received: April 5, 2017
Accepted: November 8, 2018

How to cite: Paixão, D. J. M. R.; Brabo, M. F.; Soares, L. M. O.; Campelo, D. A. V. and Veras, G. C. 2019. Optimal feeding frequency for *Heros severus* (Heckel, 1840), an Amazon ornamental fish. Revista Brasileira de Zootecnia 48:e20170055.
<https://doi.org/10.1590/rbz4820170055>

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ABSTRACT - The influence of feeding frequency on growth performance, batch uniformity, and survival rate of severum (*Heros severus*) larvae and juveniles was investigated in two experiments. In the first, 200 five-day-old severum larvae with 3.20 ± 0.31 mg and 6.20 ± 0.39 mm were randomly distributed into 20 aquaria (1 L) and fed 500 *Artemia* nauplii larvae day^{-1} for 15 days. In the second, 120 severum juveniles, 178.19 ± 33.59 mg and 1.82 ± 0.09 cm, were randomly distributed into 15 aquaria (300 L) and hand-fed a commercial diet (400.0 g kg^{-1} crude protein and 21.2 kJ g^{-1} gross energy) until apparent satiety for 30 days. For both experiments, feeding frequencies of one, two, three, four, and five meals day^{-1} were evaluated. We used four replicates for the first experiment and three for the second. At the end of both experiments, survival rate and batch uniformity were unaffected by the feeding frequency. Severum larvae fed three, four, and five meals day^{-1} showed higher final weight, weight gain, and specific growth rate, but only the larvae fed five meals day^{-1} showed higher final length and length gain than those fed once and twice day^{-1} . Severum juveniles fed two, four, and five meals day^{-1} showed higher final weight, weight gain, and specific growth rate. Growth performance parameters of final length and length gain were not affected by feeding frequencies. Thus, we recommend that the optimal feeding frequency for severum larvae fed *Artemia* nauplii is three meals day^{-1} and for juvenile severum fed a commercial diet, the optimal frequency is two meals day^{-1} .

Keywords: Amazon fish, food management, growth performance, nutrition, ornamental fish

Introduction

The practice of cultivating ornamental fish has been growing since the 1980s, with great contributions to world exports. Since 1985, the value of international ornamental trade exports increased at an average of approximately 14% per year until 1996 (FAO, 2000). In Brazil, the international ornamental fish trade is essentially restricted to the Amazon region (Pelicice and Agostinho, 2005), and wild-caught ornamental fish are potentially one of the few sustainable resources in the Amazon basin (Gerstner et al., 2006). However, due to international pressure to end wild-caught ornamental fishing practices, the commercial cultivation of these fishes has been increasing on the world stage.

Among the species that contribute to the diversity of Amazonian ornamental fauna, severum (*Heros severus*) has great potential for aquaria life because of its calm behaviour, fast adaptation to new environments, and relatively easy reproduction (Alishahi et al., 2014; Veras et al., 2016). This fish also has a great commercial appeal due to its vibrant olive green to yellow colour with shades of orange

and red. In its natural environment, it is associated with densely vegetated areas and feeds small invertebrates and plant material (Mérigoux et al., 1998; Alishahi et al., 2014; Veras et al., 2016).

Fish growth is determined by several factors, such as food quality, feeding frequency, feed intake, and the ability to absorb nutrients (Xie et al., 2011). Among these, feeding frequency plays a major role in regulating feed intake and feed waste (Wang et al., 2007; Xie et al., 2011). Most studies have confirmed that there should be an optimal feeding frequency for most fish species, especially in the early life stages (Mollah and Tan, 1982; Xie et al., 2011). Over-feeding increases costs and accumulates waste that adversely affects water quality (Biswas et al., 2006). On the other hand, if fish are fed insufficiently, growth and survival are reduced, and batch uniformity variation is increased (Jobling, 1983; Wang et al., 1998; Lawrence et al., 2012).

The determination of an optimum feeding frequency depends on the fish species, development stage, and culture system (Lee et al., 2000; Cho et al., 2003). This suggests that the effect of feeding frequency on the growth performance of fish should be analysed in detail before practical suggestions are made (Oh and Maran, 2015). Thus, in this study, we aimed to investigate the effects of different feeding frequencies on growth performance in severum larvae and juveniles.

Material and Methods

The effect of feeding frequency on severum larvae and juveniles was analysed through two consecutive experiments, both conducted in Bragança (01°03'13" S and 46°45'56" W), PA, Brazil. Research on animals was conducted according to the institutional committee on animal use (case no. 7656100517).

During the feeding trials of both experiments, water temperature and dissolved oxygen were measured daily with a thermometer and a digital oximeter (Lutron DO -5510, São Paulo, SP, Brazil), respectively. Total ammonia and pH were measured every two days with a multi-parameter meter (HI 3512, Hanna Instruments, Barueri, SP, Brazil). The concentration of unionised ammonia (NH₃) was calculated using the formula below: Unionised ammonia = total ammonia / $1 + 10^{((0.0902 - \text{pH}) + [2730 / (273.2 + \text{temperature } ^\circ\text{C})])}$.

The larvae used in this study were hatched in the laboratory under controlled environmental conditions. Five days after hatching, 200 severum larvae were randomly distributed to 20 1-L aquaria (10 larvae L⁻¹). The five different feeding frequencies were randomly assigned and consisted of four replicates of 10 larvae L⁻¹, with a mean initial body weight and length of 3.20±0.31 mg and 6.20±0.39 mm (mean±SD), respectively.

The feeding frequency treatments were: one meal at 08:00 h, two at 08:00 and 17:00 h, three at 08:00, 12:00, and 16:00 h, four at 08:00, 11:00, 14:00, and 17:00 h, and five at 8:00, 10:00, 12:00, 14:00, and 16:00 h. The severum larvae were fed 500 *Artemia* nauplii larvae⁻¹ day⁻¹, for 15 days. Every day, one hour after the last feeding, the aquaria were siphoned to ensure the maintenance of water quality, exchanging one third of the water volume.

For the second experiment, 120 severum juveniles with a mean initial weight and length of 178.19±33.59 mg and 1.82±0.09 cm (mean±SD), respectively, were randomly distributed into 15 300-L aquaria at a density of eight fish aquarium⁻¹. A completely randomised design was used, with five treatments and three replicates. During the experimental period, fish in all feeding treatment groups were hand-fed a commercial diet (114.3 g kg⁻¹ moisture, 400.0 g kg⁻¹ crude protein, 92.4 g kg⁻¹ crude lipid, 80.1 g kg⁻¹ ash, and 21.2 kJ g⁻¹ gross energy) until apparent satiety for 30 days. The feeding schedules were the same as those from the larvae trial. The aquaria were siphoned weekly for water renewal and removal of feces, exchanging one third of the water volume.

At the end of both trials, following 12 h of starvation, all fish from each aquarium unit were counted and weighed on a precision scale (model MB45 Toledo® 0.0001 g), and their standard lengths were measured with a digital caliper (model series 500 Absolute Coolant Proof® of 0.0001 m). Growth performance variables were calculated as follows: weight gain (WG = final weight - initial weight); specific growth rate (SGR = [(ln final weight - ln initial weight) × Δt⁻¹] × 100); length gain (LG = final length - initial length); and size uniformity (U = (N±20% × Nt⁻¹) × 100), in which U = uniformity in

weight or length; $N \pm 20\%$ = number of animals with weight or length varying $\pm 20\%$ from the average in each experimental unit; and N_t = total number of animals within each experimental unit at the end of the experimental period.

The mortality in both experiments was evaluated daily. Individuals were considered dead when spontaneous movements or responses to mechanical stimuli were absent. Any dead fish in the aquaria were removed and counted to calculate the survival rate (SR = (final number of animals / initial number of animals) \times 100).

Results were given as mean \pm SD. The data for both experiments were subjected to Shapiro-Wilk and Levene tests to verify the normality of errors and the homogeneity of variances, respectively. After this, data from each experiment were subjected to one-way analysis of variance (ANOVA) followed by Tukey's test ($P < 0.05$), to compare the means when differences occurred. The uniformity of larval and juvenile length and juvenile survival did not appear normal, even after transformation. Therefore, for these variables, a Kruskal Wallis test was employed. All statistical analyses were performed with Statistica 8.0 for Windows (Stat Soft 2004). The statistical model that represents the completely randomised experimental design is presented as follows:

$$Y_{(i)(j)} = \mu + t_{(i)} + e_{(i)(j)}$$

in which (i) is the number of treatment levels; (j) is the number of replicates; $Y_{(i)(j)}$ is the value of the variable tested under the given treatment level; μ is the overall average of the experiment for the analysed variable; $t_{(i)}$ is the effect of a determined level of treatment; and $e_{(i)(j)}$ is the random error.

Results

There was no effect of feeding frequency on the water quality variables ($P > 0.05$) in both experiments (Table 1).

There was no influence of feeding frequency on survival rate and on weight and length uniformity of severum larvae ($P > 0.05$). However, the growth performance was affected by feeding frequency ($P < 0.05$). Severum larvae fed three, four, and five meals day^{-1} showed higher final weight, weight gain, and specific growth rate than those fed once and twice day^{-1} . The same was observed for the final length and length gain, but fish fed five meals day^{-1} were not significantly longer than fish fed three and four meals day^{-1} (Table 2).

Survival rate, weight and length uniformity, final length, and length gain of severum juveniles were not affected ($P > 0.05$) by the different feeding frequencies. On the other hand, final weight, weight gain, and specific growth rate were higher ($P < 0.05$) for fish fed two, four, and five meals day^{-1} than those fed one and three meals day^{-1} (Table 3).

Table 1 - Water quality variables of *Heros severus* larvae and juveniles fed at different feeding frequencies

Feeding frequency (meals day^{-1})	Life stage	Temperature ($^{\circ}\text{C}$)	DO (mg L^{-1})	pH	UA (mg L^{-1})
One	Larva	28.13 \pm 0.47	6.78 \pm 0.18	6.16 \pm 0.17	0.0018 \pm 0.0013
	Juvenile	28.80 \pm 0.17	7.21 \pm 0.06	6.29 \pm 0.35	0.0018 \pm 0.0011
Two	Larva	28.51 \pm 0.37	6.79 \pm 1.41	6.17 \pm 0.13	0.0023 \pm 0.0011
	Juvenile	28.90 \pm 0.15	7.24 \pm 0.06	6.21 \pm 0.16	0.0019 \pm 0.0012
Three	Larva	28.37 \pm 0.17	6.74 \pm 1.59	6.10 \pm 0.22	0.0024 \pm 0.0014
	Juvenile	28.70 \pm 0.35	7.25 \pm 0.06	6.17 \pm 0.16	0.0019 \pm 0.0008
Four	Larva	28.61 \pm 0.34	6.74 \pm 1.86	6.11 \pm 0.16	0.0019 \pm 0.0009
	Juvenile	28.60 \pm 0.46	7.18 \pm 0.15	6.15 \pm 0.18	0.0018 \pm 0.0006
Five	Larva	28.23 \pm 0.72	6.73 \pm 2.02	6.15 \pm 0.16	0.0018 \pm 0.0009
	Juvenile	28.70 \pm 0.31	7.15 \pm 0.010	6.18 \pm 0.18	0.0017 \pm 0.0005
P-value	Larva	0.2328	0.9694	0.8588	0.1895
	Juvenile	0.2851	0.8664	0.9449	0.9876

DO - dissolved oxygen; UA - unionised ammonia.

Table 2 - Performance of *Heros severus* larvae fed at five different feeding frequencies for 15 days

Variable	Feeding frequency (meals day ⁻¹)					P-value
	One	Two	Three	Four	Five	
FW (mg)	43.78±1.14b	49.19±7.05b	60.33±2.20a	62.42±4.65a	59.33±4.60a	0.0001
WG (mg)	40.58±1.14b	45.99±7.05b	57.13±2.20a	59.22±4.65a	56.13±4.60a	0.0001
SGR (% day ⁻¹)	17.44±0.17b	18.17±0.92b	19.57±0.24a	19.79±0.50a	19.45±0.52a	0.0000
FL (mm)	14.48±0.20b	14.67±0.62b	15.12±0.27ab	15.37±0.55ab	16.00±0.26a	0.0009
LG (mm)	8.28±0.20b	8.47±0.62b	8.92±0.27ab	9.17±0.55ab	9.80±0.26a	0.0009
UW (%)	70.00±14.00a	75.00±10.00a	89.00±8.00a	79.00±19.00a	88.00±12.00a	0.1622
UL (%)	100.00±0.00a	100.00±0.00a	100.00±0.00a	98.00±0.05a	100.00±0.00a	0.4380
SR (%)	98.00±5.00a	100.00±0.00a	92.50±10.00a	93.00±10.00a	90.00±14.00a	0.5286

FW - final weight; WG - weight gain; SGR - specific growth rate; FL - final length; LG - length gain; UW - uniformity of weight; UL - uniformity of length; SR - survival rate.
Values (mean±SD; n = 4) in the same row followed by different letters are different by the Tukey test (P<0.05).

Table 3 - Performance of *Heros severus* juveniles fed at five different feeding frequencies for 30 days

Variable	Feeding frequency (meals day ⁻¹)					P-value
	One	Two	Three	Four	Five	
FW (mg)	576.36±30.30b	700.66±7.24a	642.61±9.43b	685.40±60.04a	663.92±10.66a	0.0049
WG (mg)	398.16±30.30b	522.46±7.24a	464.41±9.43b	507.20±60.04a	485.72±10.66a	0.0001
SGR (% day ⁻¹)	7.82±0.35b	9.13±0.07a	8.55±0.10b	8.96±0.57a	8.77±0.11a	0.0034
FL (mm)	23.78±0.21a	24.89±1.19a	24.48±0.24a	24.48±0.35a	24.00±0.20a	0.2174
LG (mm)	5.58±0.21a	6.69±1.19a	6.28±0.24a	6.28±0.35a	5.80±0.20a	0.2174
UW (%)	88.00±13.00a	86.00±14.00a	79.00±19.00a	96.00±7.00a	83.00±14.00a	0.6858
UL (%)	100.00	100.00	100.00	100.00	100.00	-
SR (%)	100.00±0.00a	96.00±7.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	0.4516

FW - final weight; WG - weight gain; SGR - specific growth rate; FL - final length; LG - length gain; UW - uniformity of weight; UL - uniformity of length; SR - survival rate.
Values (mean±SD; n = 3) in the same row followed by different letters are different by the Tukey test (P<0.05).

Discussion

Regardless of the feed frequency used, the results of water quality parameters remained within that recommended for the species, which is similar to a study with *H. severus* larvae (Veras et al., 2016). These results confirm that the storage density and management used in the feeding and cleaning of the experimental units were efficient and did not damage the water quality parameters.

The initial stages of the fish life cycle are considered the most critical in the production system. For many fish species, increasing feeding frequency has been demonstrated to reduce mortality, control size variation, and reduce the stress and labour costs related to grading (Wang et al., 1998; Dou et al., 2000; Costa-Bomfim et al., 2014). However, in the present study, survival rate and size uniformity of severum larvae and juveniles were not affected by feeding frequency. Increased mortality and size variations are induced by competition or hierarchy behavior, which are more accentuated when there is significant size heterogeneity among fish (Hayashi et al., 2004; Baloi et al., 2016). The small ranges of variation in the initial body weights and lengths of severum larvae and juveniles, in addition to the adequate stocking density, may explain the lack of influence of feeding frequency on the survival rate and batch uniformity of fish. In addition, the survival results observed in the present study can also be explained by the high quality of the feed offered in the two stages of fish life, even when the feed was offered at a reduced frequency.

In the present study, severum larvae fed three meals day⁻¹ had growth performance similar to larvae fed four and five meals day⁻¹, and yet displayed significantly higher growth than those fed one and two meals day⁻¹. This suggests that the appropriate feeding frequency is advantageous to growth and development of fish larvae. Larvae may have more opportunities to obtain enough feed at higher feeding frequencies than at lower feeding frequencies, leading to a higher growth rate (Xie et al., 2011). In a similar study, hybrid sunfish larvae (*Lepomis cyanellus* × *L. macrochirus*), fed to satiation three and four times daily, showed significantly greater weight gain and specific growth rates than fish fed once daily (Wang et al., 1998). Moreover, specific growth rates of large yellow croaker (*Pseudosciaena crocea*) larvae, fed two or four meals day⁻¹, were significantly lower than those fed eight and twelve meals day⁻¹ (Xie et al., 2011).

The effects of feeding frequency on fish growth are also related to the size of the stomach and intestines. Therefore, feeding frequency is also related to gastric evacuation time (Riche et al., 2004). Species with larger stomachs require lower feeding frequencies to achieve maximum growth (Pillay and Kutty, 2005), since the gastric evacuation time will be longer. Feeding at intervals shorter than the time required for the return of appetite can lead to gastric overload, resulting in reduced nutrient absorption efficiency. On the other hand, two or more meals are usually recommended for omnivorous fish (Pannevis and Earle, 1994; Wang et al., 1998; Dwyer et al., 2002), which have smaller stomachs and longer intestines compared to carnivorous fish (Costa-Bomfim et al., 2014) and, therefore, present rapid gastric emptying, as shown in a study with Nile tilapia (Riche et al., 2004). Larvae have not completed their digestive system formation and normally need higher feeding frequencies. However, after the complete development of the digestive system, in the fingerling and juvenile life stages, the feeding frequency can be reduced.

Although the digestive capacity of fish is lower in the initial stages of development, growth rate is higher than those observed in other life phases (Xie et al., 2011). Therefore, a greater number of daily meals may promote better growth for larvae and fingerlings, since there is a constant need of nutrient supply for the development of these individuals. On the other hand, an exaggerated number of meals can reduce the amount of food offered during each feeding period, which may create more disputes and hamper the development of these organisms (Hayashi et al., 2004). A high feeding frequency may also decrease the passage time of the feed in the gastrointestinal tract, which can negatively affect the activity of the digestive enzymes and, consequently, the performance of the fish, promoting a higher excretion. A high feeding frequency can also lead to an increase in production costs due to a greater need for labor and greater waste of food, which is one of the most expensive items in aquaculture (Biswas et al., 2006; Zeytin et al., 2016).

As feed is the single most significant cost involved in aquaculture operations, there is emphasis on carrying out farming by considering the maximum feed conversion into fish growth (Baloi et al., 2016). This contributes to a more economical and environmentally friendly production, since over-feeding increases costs and nitrogen waste. Nitrogen waste production in fish farms is directly affected by the feeding regime (Azevedo et al., 1998), which has an inverse correlation with feed utilisation by fish and can be minimised by regulating feeding frequency (Wang et al., 2007). However, the different feeding frequencies tested in experiments with severum did not influence water quality. It is likely that the water quality variables remained within the recommended range for the species, regardless of the feeding frequency tested, since the partial water changes were carried out daily and there was caution to not waste food. Therefore, it may be possible to reduce feeding frequency for severum larvae and juveniles without adversely affecting growth performance, size uniformity, or survival rate, thereby improving profitability through decreased labour costs.

Conclusions

The results of the present study suggest that three meals day⁻¹ is the optimum feeding frequency for severum larvae cultured with *Artemia* nauplii and two meals day⁻¹ is the optimum feeding frequency for severum juveniles.

Acknowledgments

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Pró-Reitoria de Pesquisa e Pós-Graduação of the Universidade Federal do Pará, for financial support.

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