Spanish Journal of Agricultural Research
18 (3), e0608, 11 pages (2020)
eISSN: 2171-9292
https://doi.org/10.5424/sjar/2020183-15652
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)

RESEARCH ARTICLE

OPEN ACCESS

Stocking density influences common carp larval development. Can restocking processes activate compensatory growth consequent to previous high stocking density?

Jonas H. S. Motta (Motta, JHS)[1], Leonardo S. Glória (Glória, LS)[2], Marcelo F. Polese (Polese, MF)[3], André B. de Souza (de Souza, AB)[3], Manoel A. C. Neto (Neto, MAC)[2] and Manuel V. Vidal Júnior (Vidal Júnior, MV)[2]

[1]Embrapa Pesca e Aquicultura, Palmas - Tocantins, Extension of Av. NS 10, intersection with Av. LO 18 Sentido Norte Loteamento Água Fria, Palmas, TO 77008-900, Brazil. [2]Northern Fluminense State University Darcy Ribeiro, Agricultural Sciences and Technologies Center (ASTC), Av. Alberto Lamego 2000, Parque California, Campos dos Goytacazes, Rio de Janeiro 28013-602, Brazil. [3]Federal Institute of Espírito Santo, Rua Augusto Costa de Oliveira, 660, Campus Piúma, Espírito Santo 29285-000, Brazil.

Abstract

Aim of study: To analyse the effect of stocking density on common carp larvae production. Since stocking density is one of the most important variables in recirculating aquaculture system, it is fundamental to understand its implication on fish larval development.

Area of study: Brazil

Material and methods: In an initial trial over a 30-day period, 18,000 *Cyprinus carpio* larvae were subjected to eight different stocking densities (5, 10, 15, 20, 25, 30, 35 and 40 larvae/L). In a second trial over a 15-day period, the larvae subjected to the 40 larvae/L treatment were selected according to size and 360 of them were subjected to restocking processes at a density of 5 larvae/L, in order to evaluate possible compensatory growth, while those subjected to the 5 larvae/L treatment were likewise selected according to size and were distributed at the same stocking density (5 larvae/L), to be the control treatment during the restocking process.

Main results: The larvae kept at the density of 5 larvae/L showed better growth and development. Increased heterogeneity of the concomitant batch was observed with higher stocking density. Restocking at low density (5 larvae/L), for larvae that had previously been kept at high density (40 larvae/L), caused partial compensatory growth, with an increase in the specific growth rate. Increasing the density caused increased productivity up to the density level of 25 larvae/L, but from then on there was no significant difference ($p > 0.05$).

Research highlights: Carp larvae reared at high densities need to be restocked during rearing in order to avoid the "shooting" problem.

Additional keywords: recirculating aquaculture system; fish larviculture; intraspecific competition; productivity; aquaculture.

Abbreviations used: AIC (Akaike information criterion); AICc (Akaike information criterion corrected); RAS (recirculating aquaculture system)

Authors' contributions: Conceived, designed and performed the experiments: JHSM and MVVJ. Analyzed the data: LSG. Contributed reagents/materials/analysis tools: MFP and ABS. Wrote the paper: JHSM, LSG and MACN.

Citation: Motta, JHS; Glória, LS; Polese, MF; de Souza, AB; Neto, MAC; Vidal Júnior, MV (2020). Stocking density influences common carp larval development. Can restocking processes activate compensatory growth consequent to previous high stocking density? Spanish Journal of Agricultural Research, Volume 18, Issue 3, e0608. https://doi.org/10.5424/sjar/2020183-15652

Received: 26 Aug 2019. **Accepted:** 27 Jul 2020.

Funding agencies/institutions
Federal Institute of Espírito Santo (IFES), Campus Piúma, Espírito Santo, Brazil

Competing interests: The authors have no competing interests.

Correspondence should be addressed to Manoel A. C. Neto: m.adrianocruz@gmail.com

Introduction

Larviculture for the common carp (*Cyprinus carpio*) follows the usual practices for fish production in South America, *i.e.* a semi-intensive system in earthen ponds. In this, nutrition for larvae that are already at the open-mouthed stage is based on ingestion of natural food obtained through fertilization of the cultivation pond water (Portella *et al.,* 2014). This technical approach gives rise to losses during the initial phase (until a weight of 1 g is reached) that have been estimated at 50-90% (Jelkić *et al.,* 2012).

These losses can be related to variations in the water quality of the earthen ponds that are directly related to the climatic conditions. These variations directly affect the water environment and consequently the nutrition of the larvae and, as reported by Feldlite & Milstein (1999), this influences larval growth, development and survival. Indoor systems for production of larvae are an alternative for improving survival (Jomori *et al.,* 2005; Motta *et al.,* 2019), especially during months in which climatic conditions are poor.

To compensate for the high costs involved in indoor fish production, farmers usually use high stocking densities. The stocking density is a fundamental factor in the production of aquatic organisms, and it has a direct influence on the behavior and development of fish (Joaquim *et al.,* 2014; Bosworth *et al.,* 2015; Yarahmadi *et al.,* 2015).

One problem in working with high cultivation densities is that the larvae may present low growth rates, given the competition for space and food, along with poor feed utilization (Calabrese *et al.,* 2017). On the other hand, if this density is decreased, the larvae may show compensatory growth. Ali *et al.* (2003) described compensatory growth as an accelerated phase of growth of an animal that occurs when normal conditions are restored. This feature has already been observed in several animals, including fish. Moreover, according to the same authors (Ali *et al.,* 2003), several studies have shown that compensatory gains may be present in different manners: a) total compensation, *i.e.* the animals that are deprived of food are able to reach the same size as the control group after feeding; b) partial compensation, *i.e.* the animals do not reach the size but show an acceleration in the rate of growth or better feed conversion; or c) overcompensation, *i.e.* the animals reach a greater size than that of those that are fed normally.

The objective of this study was to analyze the implications of different stocking density levels on the development, compensatory growth and productivity of common carp larvae that were raised in a closed indoor system.

Material and methods

Location, cultivation system, maintenance and water quality parameters

The experiment was carried out during the months of July and August in Rio de Janeiro, Brazil (22°27’46” S; 42°39’10” W). An 84 m2 (12 × 7 m) heated greenhouse with masonry walls and ceiling cover-coated with Styrofoam was used. Inside the greenhouse, a recirculating aquaculture system (RAS) was designed for fish larvae production. The RAS was composed of 32 rectangular plastic tanks, of capacity 25 L each, with an internal drainage system.

The filtering for the tanks consisted of a mechanical filter (acrylic fiber and expanded clay) and a biological filter (fiberglass rings), which together added 300 L, thus totaling an 1100 L system. The pump used in the system had a capacity of 4900 L/h, and the pumping rate in the cultivation tanks was adjusted to a low rate of approx. 0.35 L/min, so that the entire volume of the tank was circulated approx. 20 times per 24 h period. To avoid food losses (primarily losses of *Artemia nauplii*) screens of mesh size 80 µm were placed at the outflow of every tank. The water system included ultraviolet filtering using a 36-watt light bulb for elimination of parasites. To illuminate the area in which the experiment was carried out, four five-watt light bulbs were used during a 12 h period.

The tanks were siphoned daily in order to eliminate food remains and feces and to maintain the water quality. The mechanical filter was siphoned to remove solid particles. The water exchange was calculated in such a way as to not exceed 10% of the total volume of the system per day. The dead larvae were removed daily with the aid of a plastic pipette. On the first day, these were replaced by larvae from the same spawning that had been kept until that time in tanks under identical conditions, given that we had assumed that occurrences of mortality would be due to stress caused by counting the individuals, rather than as an effect of the treatment. From the second day on, the dead larvae were removed but not replaced, and these were counted in order to adjust the amount of food given. The water volume in the tanks was also adjusted concomitantly by lowering the height of the drain pipe (internal drain), in order to maintain the density as close as possible to that of the proposed treatment.

The water quality parameters were monitored as follows: dissolved oxygen and temperature daily (YSI F-1550A); pH three times per week (YSI F-1100), ammonia (Hanna HI83203) and electrical conductivity (Instrutherm 00929) weekly; and hardness (titration method) every two weeks. All the water quality analyses were done at 17:00.

Larvae and design

For this experiment, 18,000 larvae of the common carp were used. These were obtained from natural spawning and were kept in incubators at the reproduction laboratory until the time when the swim bladder became inflated and the mouth opened. In the present experiment, it was observed that this occurred four days after fertilization.

In order to obtain the initial weight and total length, 20 larvae were measured and weighed when they began to swim (inflation of the swim bladder). The values obtained were 1.30 mg ± 0.03 and 6.0 mm ± 0.10 for the initial wet weight and total initial length, respectively.

The experimental design was completely randomized, with eight treatments and four repetitions, and each tank was considered to be one experimental unit. The treatments were 5, 10, 15, 20, 25, 30, 35 and 40 larvae/L.

Feeding protocol

The fish were fed four times per day, at 7:00, 10:00, 13:00 and 17:00. For the first three days, only *A. nauplii* was offered (600 specimens per larva per day). On the fourth day onwards, after feeding the fish larvae with *Artemia* for 30 minutes, 0.3 g of a commercial fish food (65 g/kg crude lipid, 60 g/kg crude fiber, 110 g/kg crude ash, 360 g/kg crude protein, and 100 g/kg moisture) were offered.

The commercial fish food was firstly crushed and then sieved through a 200-400 µm mesh. The quantity offered was not changed among the treatments, because we assumed that the main food source for the fish larvae was *A. nauplii* (Jomori *et al.,* 2005; Fosse *et al.,* 2018) and that the commercial fish food was only an addition (Motta *et al.,* 2019). The aim in offering this food was to ensure that all the larvae would have enough food, thereby minimizing competition among them. The quantity of *A. nauplii* offered was doubled every seven days and the feeding protocol was adapted from Motta *et al.* (2019).

Growth, development, productivity and survival

After 30 days, all the fish larvae were counted in order to obtain survival data. For the data on the final length, growth, final weight, gain in weight and specific growth rate, ten fish larvae from each experimental unit were measured using a digital caliper (Western 6” (150 mm) ± 0.01 mm) and were weighed on an analytical balance (Shimadzu AUX 220 ± 0.001 g).

The following equations were used to calculate the variables: Survival (S) = (Final number of fish/Initial number of fish) × 100; Weight gain (WG) = Final average weight – Initial average weight; Growth (G) = Final average length – Initial average length; Specific growth rate (SGR) = ((ln final length – ln initial length)/Total days of cultivation) × 100.

The productivity was calculated based on the total quantity of fish produced in the experimental units. This was expressed as the average number of larvae produced per liter in the different treatments.

Compensatory growth

After the end of the stocking density phase, a second phase was developed in order to observe compensatory growth. Two treatments were implemented: a control treatment and a restocking treatment. In the control treatment, 397 surviving larvae from the 5 larvae/L treatment of the stocking density experiment were measured and selected according to size. From these, 360 larvae with an average length of 19.61 mm ± 0.7 mm and weight of 0.0698 g ± 0.0011 g (these values were taken as the initial average length and weight, respectively, for the control treatment) were stocked in three 25 L tanks, again at a density of 5 larvae/L. In the restocking treatment, 2263 larvae originating from the 40 larvae/L treatment of the stocking density experiment were measured and selected according to size. From these, 360 larvae with an initial average length of 14.84 mm ± 0.75 mm and weight of 0.0404 ± 0.0016 g (these values were taken as the initial average length and weight, respectively, for the restocking treatment) were restocked in three 25 L tanks at a density of 5 larvae/L. The cultivation system adopted was the same as used during the first phase (stocking density phase).

The same feeding protocol as used the first phase was adopted for the second phase. The initial quantity of *A. nauplii* offered was 1200 specimens/larvae, and this quantity was doubled after seven days.

Feeding costs

The production cost was calculated based only on the amounts spent on *Artemia* and fish food. The costs of the *Artemia* cysts and commercial fish food per kilogram were US$ 462.00 and US$ 16.84, respectively. The quantity of *Artemia* used for each treatment is mentioned in the item “Material and Methods – Feeding protocol”, and the cost of the *Artemia* was a function of the feeding protocol with the cost per kg of *Artemia*. The amount of commercial fish food offered was the same in all the treatments, and thus the cost of this feed was the same for all treatments. The quotation for the United States dollar on the day of the purchase of this input was R$ 4.20 = US$ 1.00.

Statistics

The variables that were measured directly from the fish larvae, *i.e.* growth, weight gain, final length and cost of production, were analyzed using the mixed-model methodology, based on the following statistical model (Littell *et al.,* 1998):

$$Y_{ijk} = \mu + \alpha_i + \alpha_{j(i)} + e_{k(ij)} \quad (1)$$

In this equation, Y_{ijk} is the measurement made on the k-th larva, in the j-th aquarium, which received the i-th treatment; α_i is the fixed effect of the i-th treatment and $\alpha_{j(i)}$ is the random effect of the j-th aquarium on the i-th treatment. This expected effect is distributed with a

0 average and an σ^2_a variance. The term $e_{k(ij)}$ represents the normal, independently distributed supposed random error with 0 average and σ^2 variance. The effect of the treatments was tested with the estimate of the σ^2 variance as the denominator. A presupposition of homoscedasticity for σ^2 was verified using a simple model with only one variance for the different treatments and a model containing heterogeneous variances for the different treatments.

The Akaike´s (1974) Information Criterion (AIC) was calculated, with correction using finite samples or AICc (Sugiura, 1978), in accordance with the recommendations of Burnham & Anderson (2004). The most likely was taken to be the one that presented the highest probability of verisimilitude and the parsimony criterion for the degree of parameterization of the models. The variables that related to concentrations, specific growth rate and survival were transformed to fit within the normality criterion. The transformed variable, $Y'_{ijk} = 2\arcsin\sqrt{Y_{ijk}}$, was then subjected to the same model as described in equation 1.

Table 1 presents the concentrations of both final length and weight at the original scale, after performing the $\hat{Y}_{i..} = 100\ [\sin \hat{Y}'_{i..}/2)]^2$ operation. In both cases (final length and weight), the MIXED procedure of the SAS statistical software (university edition, SAS System Inc., Cary, NC, USA) was used.

The functions relating to the characteristics of final length and weight were adjusted in relation to the concentration by using the REG procedure of the SAS statistical software (university edition, SAS System Inc., Cary, NC, USA).

Results

Water quality

There were no significant differences among the treatments ($p > 0.05$). The standard deviations were seen to be low, as follows: pH: 6.31 ± 0.03; temperature (°C): 28.05 ± 0.04; O_2 (mg/L): 6.23 ± 0.04; NH_3 (mg/L): 0.0009 ± 0.0001; and conductivity (µS/cm): 528 ± 42. These results indicate that there was little variation in the parameters throughout the cultivation.

Final length and weight

The different stocking densities influenced larval development (Table 1). Higher heterogeneity was observed in the treatments with greater density (35 and 40 larvae/L). This led to wider confidence intervals for these treatments, with regard to final length and weight.

From the regression analysis, it could be seen that density had a quadratic effect on the variables of final length (p = 0.0003) and final weight (p = 0.0026). Second-degree polynomial equations that expressed the effect of stocking density on the variables of final length (Eq. 2) and final weight (Eq. 3) were obtained:

$$y = 0.01x^2 - 0.48x + 24.65\ (R^2 = 49.8\%) \qquad (2)$$

$$y = 0.24x^2 - 9.88x + 182.11\ (R^2 = 36.8\%) \qquad (3)$$

Growth, weight gain and specific growth rate

Figures 1a and 1b demonstrate that stocking density influenced carp larvae growth and weight gain ($p > 0.05$). Larvae from treatment 5 larvae/L presented homogeneous development, instead larvae from high stocking density treatments (35 and 40 larvae/L) were heterogeneous. Given the results shown in Fig. 1c regarding the average specific growth rates, it could be seen that the larvae kept at the density of 5 larvae/L presented better daily growth. Thus, there were significant differences ($p < 0.05$) for the 10, 15, 20 25 and 30 larvae/L treatments, but there were no significant difference ($p > 0.05$) for the treatments with higher densities, *i.e.* 35 and 40 larvae/L. In the treatments with higher densities, wider confidence intervals was observed. Therefore, there was no difference between these treatments and the 5 larvae/L treatment.

Table 1. Final length and weight (with their corresponding confidence intervals) for common carp larvae cultivated at different stocking densities in a recirculating aquaculture system.

Variables (larvae/L)	Final length (mm)			Final weight (mg)		
	Average	Confidence interval		Average	Confidence interval	
		Lowest	Highest		Lowest	Highest
5	22.96^{a}	21.94	23.97	142.46^{a}	120.62	164.30
10	20.55^{b}	19.33	21.76	102.57^{ab}	79.55	125.58
15	19.70^{b}	18.53	20.88	92.31^{b}	71.52	113.09
20	18.89^{b}	17.52	20.26	80.67^{b}	57.30	104.04
25	19.26^{b}	17.76	20.75	80.63^{b}	57.22	104.04
30	19.55^{b}	17.95	21.15	97.28^{ab}	58.79	135.77
35	21.84^{ab}	19.52	24.16	162.46^{ab}	82.46	242.47
40	21.20^{ab}	18.72	23.69	162.20^{ab}	62.64	261.76

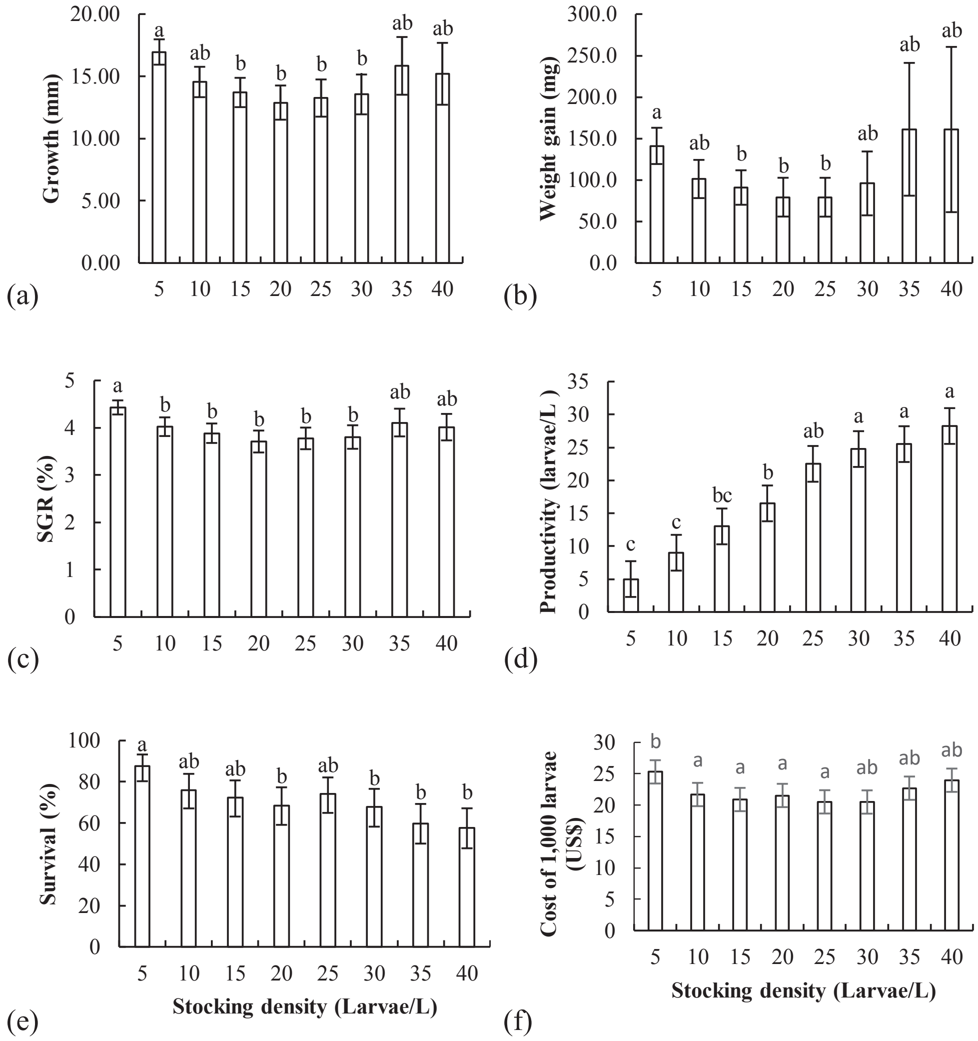

Figure 1. Means and confidence intervals of: (a) growth; (b) weight gain; (c) specific growth rate (SGR); (d) productivity; (e) survival; and (f) feeding cost, of common carp larvae that were cultivated at different stocking densities in a recirculating aquaculture system.

Growth, weight gain and specific growth rate

It was observed that the density had an effect on the survival of the larvae of these common carp (Fig. 1d). The treatment with 5 larvae/L presented a higher average than what was seen in the treatments with 20, 30, 35 and 40 larvae/L, but there was no significant difference ($p > 0.05$) in relation to the treatments with 10, 15 and 25 larvae/L.

The mortality rates throughout the cultivation period were similar in all treatments. There was an increase in mortality starting on the 9th day (Fig. 2). The eyes and fins of most of the dead larvae were found to have been eaten.

Despite the better development of the larvae that was observed in the 5 larvae/L treatment, the result regarding productivity was the opposite (Fig. 1e). Productivity increased significantly with increasing stocking density up to the level of 25 larvae/L. From this point on, no further significant increase in productivity was observed ($p > 0.05$).

Feeding cost

The feed cost was significantly lower in the 15, 25 and 30 larvae/L treatments ($p < 0.05$). On the other hand, in the 5, 10, 20, 35 and 40 larvae/L treatment, the feed cost was high (Fig. 1f).

Compensatory growth

The larvae that had previously been kept at high density (40 larvae/L) presented a higher specific growth rate regarding their length ($p < 0.05$) than what was observed for the larvae that had previously been kept at lower density (5 larvae/L), when they were restocked at low density (5 larvae/L) (Fig. 3a).

Partial compensatory gain was found (Fig. 3b). Thus, even though there was a significant difference ($p < 0.05$) in daily growth between the larvae of the restocking

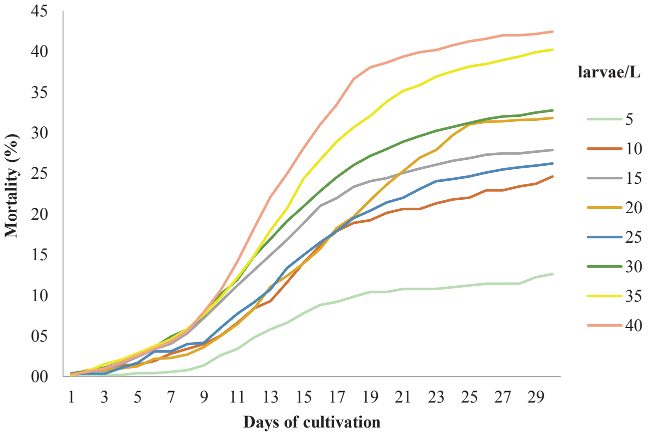

Figure 2. Evolution of mortality of common carp larvae during cultivation at eight stocking densities.

treatment and those of the control treatment, this was not enough for the fish in the two treatments to have similar final lengths.

No significant difference in the specific growth rate by weight was observed ($p > 0.05$), but it was noted that the average was slightly higher in the restocking treatment (Fig. 3c). The slight improvement in the average specific growth rate that was seen was not enough to reverse the difference in the final weight of the restocked larvae (Fig. 3d).

Survival was not affected by the treatments in this phase (94% in the control treatment and 92.5% in the restocking treatment). Thus, no significant difference ($p > 0.05$) was observed between the treatments.

Discussion

The water quality parameter values obtained for these experiments were within the range that had previously been mentioned as acceptable for carp cultivation (Boyd, 1982; Motta *et al.,* 2019). The recirculating aquaculture system adopted in our experiments proved to be efficient in maintaining the water quality parameters within the acceptable range for the duration of the experiment.

Use of live food (*A. nauplii*) as the first exogenous feeding material has been shown to be efficient for several species (Kamaszewski *et al.,* 2014; Fosse *et al.,* 2018). The feeding protocol adopted in the present experiment was an adaptation from Motta *et al.* (2019) and proved to be efficient for indoor carp larvae production. The frequency of feeding adopted (four feeds per day) is functional for rearing larvae and this is used by freshwater fish farmers. Fish larvae are animals undergoing a period of transition and need constant feeding (Portella *et al.,* 2014).

Contrary to what was observed in our experiment, some other authors did not report significant differences in the performance of fish cultivated at different densities (North *et al.,* 2006; Webb Jr. *et al.,* 2007; Salas-Leiton *et al.,* 2008). In most cases, these authors were working with

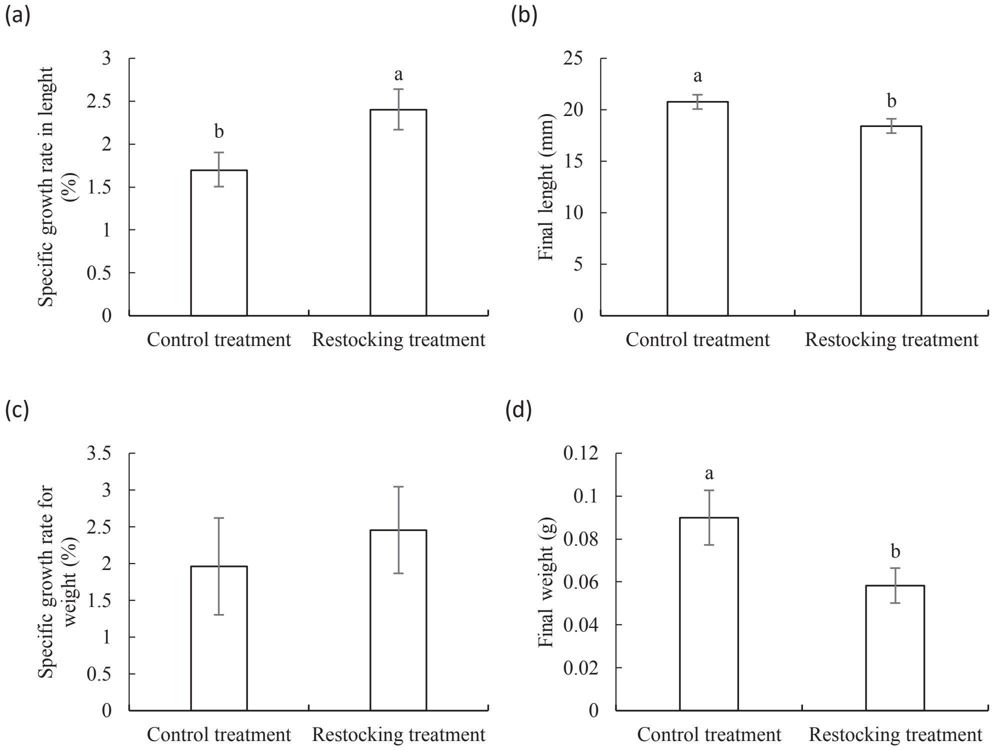

Figure 3. Means and confidence intervals of: (a) specific growth rate for length; (b) final length; (c) specific growth rate for weight; and (d) final weight, of common carp larvae that were submitted to second phases' treatments: Control or Restocking.

fish that were in their juvenile or adult phase, whereas in the present experiment the fish were at the larval phase. It is known that in fish the specific growth rate is generally higher in larvae than in the juvenile and adult forms.

High stocking densities cause chronic stress within fish cultivation (De las Heras *et al.,* 2015; Yarahmadi *et al.,* 2015), which leads to problems of high mortality and poor development (Moniruzzaman *et al.,* 2015). Different authors have come to differing conclusions regarding how the effect of the stocking density influences the development of fish. Some believed that the agglomeration effect directly interfered with environmental factors: especially pH, O_2, ammonia and nitrate (Sampaio *et al.,* 2001; Bosworth *et al.,* 2015). In the present experiment, the problem of poor water quality was avoided by using a recirculating aquaculture system. Thus, differences in mean was a consequence of another cultivation problem.

Competition for space and food is the main reason why the stocking density has an effect on the development of the fish. High density limits the access to food, thereby leading to chronic stress responses (Ardiansyah & Fotedar, 2016), cannibalism and/or development of diseases. In our experiment, the effect of stocking density on growth and development of the larvae seemed to be the result of intra-specific competition.

It is possible that increasing the stocking density decreased the efficiency of food use. Similar data were reported by Calabrese *et al.* (2017). Even though the amount of food provided for the larvae had been calculated as being sufficient for all of them, this did not ensure that every larva would eat the amount of food that had been estimated. The actual amount consumed depends mainly on the competition within the cultivation.

Another sight would be that, given that the concentration and spatial distribution of *A. nauplii* differed among the treatments, this could somehow affect the larval development. Since *A. nauplii* has the natural habit of agglomerating in a specific area, their distribution in the experimental unit would therefore favor just a few larvae. These factors probably increased the competition for food and space and had a direct influence on the homogeneity of the batch, in terms of final length and weight. Similar results were observed by Gonçalves Júnior *et al.* (2014), working with goldfish larvae (*Carassius auratus*).

Contrary to what was observed in our experiment, Ako *et al.* (2005) observed that carp were non-competitive feeders, and that stocking density did not affect their development. However, it is important to mention that these authors worked with two densities (1 fish/L and 4 fish/L), and both of these were lower than the lowest density that was tested during the present experiment (5 larvae/L). It is possible that this low density tested by Ako *et al.* (2005) affected the competition data among the fish. Moreover, these authors observed that the mortality rates were high and that most of the dead fish were partially eaten and concluded that carp are very aggressive fish.

The coefficients of determination for the variables of final length (Eq. 2) and final weight (Eq. 3) showed that these variables presented low reliability for predicting the effect of stocking density on batches of carp larvae. This was a consequence of the heterogeneity of the larvae, especially those subjected to high stocking densities.

Lack of uniformity of size of fish in shoals was reported by Vilizzi & Walker (1999). These authors mentioned that in carp shoals it is common to see a phenomenon known as "shooting", in which a few larvae grow much more than the others in the shoal. They commented that this phenomenon occurred in situations of exacerbated competition for food within the shoal. They also reported that cases of cannibalism occurred when the intra-specific competition became excessive. In the present experiment, the "shooting" problem was observed mainly in treatments with high densities.

Behavioral changes that were directly linked to higher stocking density were also reported by Khan (1994). This author mentioned that at high densities, some of the fish presented aggressive and domineering behavior. Although no domineering behavior was observed during the present experiments, it was observed the bodies of some dead fish had been partially eaten (e.g. the eyes and fins) and this finding is in line with what as reported by Khan (1994) and Ako *et al.* (2005).

On the other hand, Van de Nieuwegiessen *et al.* (2008; 2009) suggested that at high stocking densities, juveniles of the African catfish (*Clarias gariepinus*) did not demonstrate agonistic behavior. But when stocking densities were reduced to low levels, juveniles began to display agonistic behavior. This situation is common for species that demonstrate hierarchical behavior. For these species, when the density is low, a few fish chase all the others, thus preventing most of the fish from having access to food. In these cases, at low stocking densities, fish demonstrate heterogeneous development, because of their poor access to food. Contrary to what was observed for African catfish, heterogeneous development was observed in the present experiment when stocking densities were high and not when they were low. Moreover, no domineering behavior was not observed. Therefore, it is inappropriate to promote parallels between the behaviors of common carp and African catfish.

Some authors have reported behavioral changes even in the feeding habits of some species, when the stocking density was increased (Ako *et al.,* 2005). Paspatis *et al.* (2003), for example, working with sea bass (*Dicentrarchus labrax*), observed that with increasing density, some fish began to display nocturnal activity, whereas when they were kept at low densities, only daytime activity (the natural habit of this species) was observed. The above-mentioned authors believed that with increasing density, competition for access to the feeders increased. Thus, in order to have access to food, some individuals were motivated to change their habits to nocturnal activities.

To solve the problems of shooting and heterogeneity, a restocking strategy was tested in the present study. It was observed that the larvae in the restocking treatment had a higher daily growth rate. This period of fast growth demonstrated that developmental deficits caused by high stocking density during the first 30 days of cultivation can be reverted. For this to occur, the fish only need to be moved to a healthier environment, with less competition and better access to food.

The same findings were demonstrated by Jobling & Koskela (1996), working with juvenile rainbow trout (*Oncorhynchus mykiss*). These authors proved that highly heterogeneous growth among the fish resulted from feeding hierarchies, such that just a few of the fish consumed most of the food, while the others experienced developmental deficit. The same authors showed that, after passing through a period of feed restriction, fish with developmental deficit showed compensatory growth when the feed regime was corrected.

No dominance hierarchy was observed in the present study, but hierarchism is not the only factor that influences social interactions. Behavioral status and body condition can vary between individuals, and these factors can lead to heterogeneous growth (Jobling & Koskela, 1996; Ali *et al.,* 2003). However, as observed in the present experiment, this condition of heterogeneous growth (shooting problem) can be reversed by solving the crowding problem through a restocking strategy.

In the present study, the fish larvae in the restocking treatment were not able to match the final length of the larvae in the control treatment. This result is characteristic of partial compensatory growth. Our findings differed from those of Schwarz *et al.* (1985). These authors studied common carp juveniles under the influence of limited crude protein or energy supply. They did not observe any kind of compensatory growth, or even any increase in the daily gain, when these fish started to receive the control diet.

It is likely that in our study, full compensation was not observed because of the short cultivation time (two weeks) of this phase. In most experiments in which the fish reached full compensation, more than two weeks of re-alimentation was needed in order to achieve this (Myszkowski *et al.,* 2013; Sevgili *et al.,* 2013; Yengkokpam *et al.,* 2014). Zhu *et al.* (2005) worked with Chinese long snout catfish (*Leiocassis longirostris*) and observed that full compensatory growth was achieved after one or two weeks of food deprivation, if this was followed by four weeks of feeding. Full compensatory growth was probably observed because of the longer cultivation time.

The restocking protocol was implemented in cultivation of the turbot *Scophthalmus maximus* (Smith, 1979). Restocking strategies have not only been used to solve food competition problems: some authors have mentioned that this technique is of great importance for cultivation of the larvae of certain species. Kotani *et al.* (2011) addressed problems of cannibalism in larviculture of *Takifugu rubripes* and improvement of survival over the course of the cultivation period. They suggested that the fish larvae should undergo restocking after 21 days, with diminution of the stocking density from 5 larvae/L to 2 larvae/L.

The significant effect that stocking density had on the development of the common carp larvae in the present study was reflected in the average final lengths observed. These lengths were similar to those obtained by other authors in intensive cultivation of common carp larvae (Motta *et al.,* 2019). Alami-Durante *et al.* (1991) studied different diets for carp larvae (*C. carpio*) at a density of 77.78 larvae/L for 31 days. From their best treatment, they obtained larvae with an average final length of 34 mm and a range from 23 mm to 39 mm. These authors attributed the wide variance in this variable that occurred in some treatments (which was also seen in our experiment) to the high stocking density used.

The decreased survival that was observed in our experiment, which correlated with increases in the stocking density, was probably related to intra-specific competition. Several authors have mentioned the existence of survival problems in cultivations when the density levels are increased. Fairchild & Howell (2001) investigated what the optimal density for cultivation of juvenile winter flounders would be. They concluded that high densities (200% and 300% of the area of the pond bottom) did not affect growth but did affect survival. These authors believed that decreased survival correlated with increasing density and, as also seen in our experiment, with the stress of intra-specific competition. However, contrary to the data from the present experiment, Menezes *et al.* (2015) did not observe any effect from stocking density on mortality among fish.

Despite the inversely proportional relationship between increase stocking density and survival, even at more elevated densities (35 and 40 larvae/L), the survival of the common carp larvae when cultivated in a recirculating aquaculture system was higher than the survival reported by other authors for fish larval production in earthen ponds (Chabalin *et al.,* 1989; Jelkić *et al.,* 2012). Survival superior results in recirculating aquaculture system was probably due to better conditions propitiated by fish production in this system, such as high-quality food supply and water quality control.

It was observed that increased productivity was directly linked to increasing the stocking density, but inversely proportional to the growth and development of the fish larvae. This was also observed by Gonçalves Júnior *et al.* (2014), who reported that increased production correlated with increases in the stocking density but with decreases in fish larval growth. Productivity also influenced the feeding cost. It is known that the feeding cost may represent 70% of the total cost of production (Ali *et al.,* 2003), and this is a variable of interest for farmers. Our analysis on this variable confirmed that, regarding fish larval production, there was a limit to stocking density beyond which farmers may waste money.

In the present experiment, carp larvae cultivated at high stocking densities presented a "shooting" problem that could be resolved through restocking the larvae at a suitable stocking density. It was found that the restocked larvae developed compensatory growth, but a period longer than fifteen days was needed in order to achieve full compensation. Another sight might be that indoor larvae cultivation could yield satisfactory survival results.

Acknowledgments

The authors wish to express their thanks to Piscicultura Mario Porto Ltda. for its donation of fish larvae for the experiments, to Poytara© for its donation of commercial fish food.

References

Akaike H, 1974. A new look at the statistical model identification. IEEE Trans Automat Contrl 19 (6): 716-723. https://doi.org/10.1109/TAC.1974.1100705

Ako H, Shimizu E, Lemos K, Asano L, Tamaru C, 2005. Behavioral limitations of high density fish growout. W Aquacult 36: 25-29, 67.

Alami-Durante H, Charlon N, Escaffre AM, Bergot P, 1991. Supplementation of artificial diets for common carp (*Cyprinus carpio* L.) larvae. Aquacult 93 (2): 161-175. https://doi.org/10.1016/0044-8486(91)90215-S

Ali M, Nicieza A, Wootton RJ, 2003. Compensatory growth in fishes: a response to growth depression. Fish Fish 4 (2): 147-190. https://doi.org/10.1046/j.1467-2979.2003.00120.x

Ardiansyah A, Fotedar R. 2016. Water quality, growth and stress responses of juvenile barramundi (*Lates calcarifer* Bloch), reared at four different densities in integrated recirculating aquaculture system. Aquacult 458: 113-120. https://doi.org/10.1016/j.aquaculture.2016.03.001

Bosworth B, Ott B, Torrans L, 2015. Effects of stocking density on production traits of Channel Catfish x Blue Catfish Hybrids. N AM J Aquacult 77 (4): 437-443. https://doi.org/10.1080/15222055.2015.1024363

Boyd CE, 1982. Water quality management of pond fish culture. Elsevier, Amsterdam.

Burnham KP, Anderson DR, 2004. Multimodel inference: understanding AIC and BIC in model selection. Sociol Methods Res 33 (2): 261-304. https://doi.org/10.1177/0049124104268644

Calabrese S, Nilsen TO, Kolarevic J, Ebbesson LOE, Pedrosa C, Fivelstad S, Hosfeld SO, Stefansson BF, Terjesen H, Takle CIM, *et al.,* 2017. Stocking density limits for post-smolt Atlantic salmon (*Salmo salar* L.) with emphasis on production performance and welfare. Aquacul 468 (1): 363-370. https://doi.org/10.1016/j.aquaculture.2016.10.041

Chabalin E, Senhorini JÁ, Ferraz De Lima JA, 1989. Estimativa de custo de produção de larvas e alevinos. Bol Téc do CEPTA 2: 61-74.

De las Heras V, Martos-Sitcha JA, Yúfera M, Mancera JM, Martínez-Rodríguez G, 2015. Influence of stocking density on growth, metabolism and stress of thick-lipped grey mullet (*Chelon labrosus*) juveniles. Aquacul 448: 29-37. https://doi.org/10.1016/j.aquaculture.2015.05.033

Fairchild EA, Howell WH, 2001. Optimal stocking density for juvenile Winter Flounder *Pseudopleuronectes americanus*. J World Aquacult Soc 32 (3): 300-308. https://doi.org/10.1111/j.1749-7345.2001.tb00453.x

Feldite M, Milstein A, 1999. Effect of density on survival and growth of cyprinid fish fry. Aquacult Int 7 (6): 399-411.

Fosse PJ, Mattos DDC, Cardoso LD, Radael MC, Vidal Jr. MV, 2018. Duration of co-feeding on the Nishikigoi *Cyprinus carpio* larvae during weaning from live to inert food in an indoor system. Ciênc Rural 48 (4): 1-9. https://doi.org/10.1590/0103-8478cr20170579

Gonçalves Júnior LP, Mendonça PP, Pereira SL, Matielo MD, Amorim IRS, 2014. Densidade de estocagem durante a larvicultura do kinguio. Bol Inst Pesca 40 (4): 597-604. https://www.pesca.sp.gov.br/boletim/index.php/bip/article/view/1065/1043

Jelkić D, Opačak A, Stević I, Jug-Dujaković J, Safner R, 2012. Rearing carp larvae (*Cyprinus carpio*) in closed recirculatory system (RAS). Croat J Fish 70 (1): 9-17. https://hrcak.srce.hr/78877

Joaquim S, Matias D, Matias AM, Leitão A, Soares F, Cabral M, Chícharo L, Gaspar MB, 2014. The effect of density in larval rearing of the pullet carpet shell Venerupis corrugata (Gmelin, 1791) in a recirculating aquaculture system. Aquacult Res 47 (4): 1-12. https://doi.org/10.1111/are.12561

Jobling M, Koskela J, 1996. Interindividual variations in feeding and growth in rainbow trout during restricted feeding and in a subsequent period of compensatory growth. J Fish Biol 49 (4): 658-667. https://doi.org/10.1111/j.1095-8649.1996.tb00062.x

Jomori RK, Carneiro DJ, Martins MIEG, Portella MC, 2005. Economic evaluation of Piaractus mesopotamicus juvenile production in different rearing systems. Aquacult 243 (1-4): 175-183. https://doi.org/10.1016/j.aquaculture.2004.09.034

Kamaszewski M, Prasek M, Ostaszewska T, Dabrowski K, 2014. The influence of feeding diets containing wheat gluten supplemented with dipeptides or free amino acids on structure and development of the skeletal muscle of carp (*Cyprinus carpio*). Aquacult Int 22 (1): 259-271. https://doi.org/10.1007/s10499-013-9683-0

Khan MS, 1994. Effect of population density on the growth, feed and protein conversion efficiency and biochemical composition of a tropical freshwater catfish, *Mystus nemurus* (Cuviei and Valenciennes). Aquacult Res 25 (7): 753-760. https://doi.org/10.1111/j.1365-2109.1994.tb00739.x

Kotani T, Wakiwaya Y, Imoto T, Fushimi H, 2011. Improved larviculture of ocellate puffer *Takifugu rubripes* through control of stocking density. Aquacult 312 (1-4): 95-101. https://doi.org/10.1016/j.aquaculture.2011.01.001

Littell RC, Henry PR, Ammermman CB, 1998. Statistical analysis of repeated measures data using SAS procedures. J Anim Sci 76 (4): 1216-1231. https://doi.org/10.2527/1998.7641216x

Menezes C, Ruiz-Jarabo I, Martos-Sitcha J, Toni C, Salbego J, Becker A, Loro VL, Martínez-Rodríguez G, Mancera J, Baldisserotto B, 2015. The influence of stocking density and food deprivation in silver catfish (*Rhamdia quelen*): A metabolic and endocrine approach. Aquacult 435: 257-264. https://doi.org/10.1016/j.aquaculture.2014.09.044

Moniruzzaman M, Uddin KB, Basak S, Mahmud Y, Zaher M, Bai SC, 2015. Effects of stocking density on growth, body composition, yield and economic returns of monosex Tilapia (*Oreochromis niloticus* L.) under cage culture system in Kaptai Lake of Bangladesh. J Aquac Res Dev 6 (8): 1-7. https://doi.org/10.4172/2155-9546.1000357

Motta JHS, Vidal Jr MV, Glória LS, Cruz Neto MA, Silveira LS, Andrade DR, 2019. Technical and economic feasibility of food strategies in the hatchery of *Cyprinus carpio* (*Cypriniformes, Cyprinidae*) in a recirculating aquaculture system. Lat Am J Aquat Res 47 (4): 626-637. https://doi.org/10.3856/vol47-issue4-fulltext-5

Myszkowski L, 2013. Compensatory growth, condition and food utilization in barbell *Barbus barbus* juveniles reared at different feeding periodicities with dry diet. J Fish Biol 82 (1): 347-353. https://doi.org/10.1111/j.1095-8649.2012.03482.x

North BP, Turnbull JF, Ellis T, Porter MJ, Migaud H, Bron J, Bromage NR, 2006. The impact of stocking density on the welfare of rainbow trout (*Oncorhynchus mykiss*). Aquacult 255 (1-4): 466-479. https://doi.org/10.1016/j.aquaculture.2006.01.004

Paspatis M, Boujard T, Maragoudaki D, Blanchard G, Kentouri M, 2003. Do stocking density and feed reward level affect growth and feeding of self-fed juvenile European sea bass? Aquacult 216 (1-4): 103-113. https://doi.org/10.1016/S0044-8486(02)00417-9

Portella MC, Jomori RK, Leitão NJ, Menossi OCC, Freitas TM, Kojima JT, Lopes TS, Clavijo-Ayala JA, Carneiro DJ, 2014. Larval development of indigenous South American freshwater fish species, with particular reference to pacu (*Piaractus mesopotamicus*): A review. Aquacult 432: 402-417. https://doi.org/10.1016/j.aquaculture.2014.04.032

Salas-Leiton E, Anguis V, Machado M, Cañavater JP, 2008. Growth, feeding and oxygen consumption of Senegalese sole (Solea senegalensis) juveniles stocked at different densities. Aquacult 285 (1-4): 84-89. https://doi.org/10.1016/j.aquaculture.2008.08.001

Sampaio LA, Ferreira AH, Tesser MB, 2001. Effect of stocking density on laboratory rearing of mullet fingerlings, Mugil platanus (Günther, 1880). Acta Scientiarum 23 (2): 471-475. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.921.7805&rep=rep1&type=pdf

Schwarz FJ, Plank J, Kirchgessner M, 1985. Effects of protein or energy restriction with subsequent realimentation on performance of carp (*Cyprinus carpio* L.). Aquacult 48 (1): 23-33. https://doi.org/10.1016/0044-8486(85)90049-3

Sevgili H, Hoşsu B, Emre Y, Kanyılmaz M, 2013. Compensatory growth following various time lengths of restricted feeding in rainbow trout (*Oncorhynchus mykiss*) under summer conditions. J Appl Ichthyol 29 (6): 1330-1336. https://doi.org/10.1111/jai.12174

Smith PL, 1979. The development of a nursery technique for rearing turbot *Scophthalmus maximus*, from the metamorphosis to ongrowing size-Progress since 1970 by the British White Fish Authority [1976]. In: Advances in Aquaculture; Pillay TVR & Dill WA (eds), FAO, pp: 143-149. http://agris.fao.org/agris-search/search.do?recordID=XF19760124310

Sugiura N, 1978. Further analysis of the data by Akaike's information criterion and the finite corrections. Commun Stat Theor Meth A7: 13-26. https://doi.org/10.1080/03610927808827599

Van de Nieuwegiessen PG, Boerlage AS, Verreth JAJ, Schrama JW, 2008. Assessing the effects of a chronic stressor, stocking density, on welfare indicators of juvenile African catfish *Clarias gariepinus*. Appl Anim Behav Sci 115: 233-243. https://doi.org/10.1016/j.applanim.2008.05.008

Van de Nieuwegiessen PG, Olwo J, Khong S, Verreth JAJm Schrama JW, 2009. Effects of age and stocking density on the welfare of African catfish, *Clarias gariepinus* Burchell. Aquacult 288: 69-75. https://doi.org/10.1016/j.aquaculture.2008.11.009

Vilizzi L, Walker K, 1999. The onset of the juvenile period in carp, *Cyprinus carpio*: a literature survey. Envir Biol Fish 56: 93-102. https://doi.org/10.1007/978-94-017-3678-7_7

Webb Júnior KA, Hitzafelder GM, Faulk CK, Holt J, 2007. Growth of juvenile cobia, Rachycentron canadum, at three different densities in a recirculating aquaculture system. Aquacult 264 (1-4): 223-227. https://doi.org/10.1016/j.aquaculture.2006.12.029

Yarahmadi P, Miandare HK, Hoseinifar SH, Gheysvandi N, Akbarzadeh A, 2015. The effects of stocking density on hemato-immunological and serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). Aquacult Int 23 (1): 55-63. https://doi.org/10.1007/s10499-014-9797-z

Yengkopam S, Sahu NP, Pal AK, Debnath D, Kumar S, Jain KK, 2014. Compensatory growth, feed intake and body composition of Labeo rohita fingerlings following feed deprivation. Aquacult Nutr 20 (2): 101-108. https://doi.org/10.1111/anu.12056

Zhu X, Xie S, Lei W, Cui Y, Yang Y, Wootton RJ, 2005. Compensatory growth in the Chinese longsnout catfish, *Leiocassis longirostris* following feed deprivation: Temporal patterns in growth, nutrient deposition, feed intake and body composition. Aquacult 248 (1-4): 307-314. https://doi.org/10.1016/j.aquaculture.2005.03.006