Research Article

Effect of stocking density on growth, survival and condition factor in tropical gar (*Atractosteus tropicus* Gill, 1863) juveniles

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ABSTRACT. Tropical gar (*Atractosteus tropicus*) is currently exploited regionally as food and commercialized in the aquarium trade. Natural populations of *A. tropicus* may already be negatively affected by anthropogenic alteration of the areas in which it is distributed. However, it is necessary to deepen the knowledge for the optimization of its culture. *A. tropicus* cultured in three stocking densities: 100, 200, and 300 ind m^{-3} (D4, D8, and D12, respectively) were used in the present study to examine the effect on growth, survival, and condition factor. At the end of the eight-week trial, D4 was statistically superior in weight, length, and SGR compared to D12. While D8 was statistically similar to D4 and D12 in weight and length. There were no significant differences in the survival and condition of the fish. The optimum density in the production systems generates the optimal use of resources and the increase in the profitability of the system. Based on the results, it is recommendable a stocking density of 200 fish m^{-3} for an increase in the profitability of the culture infrastructure for its production as food or ornamental purposes.

Keywords: *Atractosteus tropicus*; stocking density; feeding; gar; aquaria; juvenile; tropical; aquaculture

INTRODUCTION

Tropical gar (*Atractosteus tropicus*) it is considered a living fossil. It belongs to the Lepisosteidae family, its distribution in the tropical southeastern freshwaters systems in Mexico, where it represents a food source for local communities, although it is present in Nicaragua, Guatemala and Costa Rica (Bussing, 2002; Márquez-Couturier & Vázquez-Navarrete, 2015). Its culture has been achieved in captivity where it has had accepted artificial diets, while in the wild feeds mostly on fish and micro-crustacean (Miller *et al*., 2005; Nelson *et al*., 2016). It is currently being marketed for human consumption at a local/regional scale and as an ornamental fish countrywide. However, wild populations have been strongly impacted by changes in land use due to population growth, extreme climatic conditions, pollution, and overfishing (Márquez-Couturier *et al*., 2015), as well as the introduction of invasive species such as tilapias *Oreochromis* spp. (Cassemiro *et al*., 2017). The improvement of *A. tropicus* culture can help the diversification of Mexican aquaculture and alleviate illegal fishing, which can deteriorate natural populations (Waples *et al*., 2007). Their studies focused on innovative culture techniques that have been a successful tool to increase production

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and protect native species (Pérez-Sánchez & Páramo-Delgadillo, 2008; Martínez-Cárdenas & Purser, 2011).

Stocking density is a key factor in aquaculture that has to be considered in the infrastructure planning for this activity as impacts on fish performance (Oppedal *et al.*, 2011; Riche *et al.*, 2013; Basto-Rosales *et al.*, 2019). It can have an influence in various response variables such as survival, growth and condition factor of fish, and its effects can be positive or negative depending on the species, its life stage and the system/practice adopted (Tolussi *et al.*, 2010; Tibile *et al.*, 2016). A suboptimal stocking density can have adverse effects on fish survival and growth due to competence for food and space that can lead to inadequate food intake and conversion efficiency as a result of chronic stress (Ellis *et al.*, 2002; Diana *et al.*, 2004; Ashley, 2007; Abdel-Tawwab, 2012; Akinwole *et al.*, 2014).

Several studies have found that a high stocking density can be the source of that stress either for hierarchical interactions or an excessive number of fish been raised in a tank (Bolasina *et al.*, 2006). The majority of the studies on stocking density, have found a positive effect at low stocking densities and have been focused in freshwater fish species with commercial importance such as tilapia (Osofero *et al.*, 2009; Yuan *et al.*, 2010; Mensah *et al.*, 2013; Daudpota *et al.*, 2014; Ronald *et al.*, 2014), and African catfish *Clarias gariepinus* (Van de Nieuwegiessen *et al.*, 2009; Dasuki *et al.*, 2013). These studies also have been conducted using low intensification production systems such as floating cages and ponds. A few studies have shown that a low stocking density triggers a negative hierarchical interaction (Chakraborty & Banerjee, 2010; Shubha & Reddi, 2011; Aragón-Flores *et al.*, 2014). Finding an adequate density contributes to the optimization of production systems. A balance between the resources used, a product of differentiated quality and environment impact was established, all this leads to the maximum possible profitability for the producer. Therefore, the present study aimed to examine the effect on growth, survival, and condition of *A. tropicus* cultured in 100 (D4), 200 (D8), and 300 (D12) fish m^{-3}.

MATERIALS AND METHODS

System design and general methods

A total of 81 juveniles (mean ± SD, standard deviation) 9.15 ± 0.16 cm in standard length (tip of the snout to the base of caudal fin) and 3.80 ± 0.11 g in wet weight, were allocated to a 200 L holding tank at a temperature of 26°C and a salinity of 0 g L^{-1}. Each 40 L glass tank used in the experiment had a biological platform filter

(3 mm gravel as filtration substrate) activated by continuous aeration provided via an aeration pump (Optima, Hagen®; Mansfield, Massachusetts) connected to flexible plastic tubing ending with 2.5 cm air stone diffuser. In each tank, a heater was set to maintain the desired water temperature. A 12:12 (L:D) photoperiod was provided (lights on at 08:00 h, lights off 20:00 h) by a timer-controlled cool white light 35 W (General Electric Company; Fairfield, CT, USA) producing an intensity of 4.8 μE m^{-2} s^{-1} at the water surface. Water quality for the experiment was maintained as follows: average pH 7.8 (range 7.6-8.0), dissolved oxygen >75%, total ammonia nitrogen (TAN) < 0.5 mg L^{-1}, nitrite < 0.25 mg L^{-1}, nitrate < 5 mg L^{-1}. For the determination of pH, TAN, nitrite, and nitrate, a colorimetric saltwater liquid test kit (Aquarium Pharmaceuticals; Chalfont, PA, USA.) was used. The temperature was monitored every 24 h while TAN, pH, nitrite, and nitrate were recorded every 48 h during the experiment. Tanks were inspected daily for mortalities, and any excess food and feces were siphoned to waste.

Experimental design

For this study three culture densities were tested; treatment D4 consisted in a stocking density of four individuals per tank (100 ind m^{-3}), in treatment D8 were located eight individuals per tank (200 ind m^{-3}) and in treatment D12, 12 individuals per tank (300 ind m^{-3}). During the experiment the food provided was pelleted for juvenile trout "El Pedregal" that were 1.5 mm particles, consisting of 45% protein and 16% fat, a ration rate of 2% body weight (BW) per day (dry weight pellet: wet weight fish) divided into two equally sized meals (1000 and 1500 h). The mentioned trout diet was selected because as it is similar to the actual requirements of *Atractosteus tropicus* at the age tested in the present study (Contreras, 2008; Huerta-Ortiz *et al.*, 2018). After a series of attempts, the ration rate of 2% body weight (BW) per day (dry weight pellet:wet weight fish) was chosen to achieve an ingestion rate without waste. Feeding adjustments were calculated based on the daily mortality (assigned by the previously recorded mean weight) and weekly bulk weight per tank, (the rations corresponding to mortalities were not fed to the remainder of fish).

The standard length was measured by placing the fish on a 1 mm scaled sheet covered with plastic. Wet weight was measured on an electronic scale and recorded to the nearest 0.1 g. Fish were not fed for 24 h before each weighing. Standard length and wet weight of individual fish were recorded on day zero. After eight weeks, the surviving fish were counted. Their wet weight and standard length measured individually.

These parameters were calculated according to the following formulae: survival (%) = 100 - (initial number of fish - final number of fish / initial number of fish) × 100; length gain (cm d^{-1}) = final length - initial length / experiment days; in weight gain (g d^{-1}) = final weight - initial weight / experiment days. Fulton's K was calculated as K = (W × L^{-3}) × 100, where W = wet weight (g) and L = standard length (cm). Specific growth rate (SGR) was calculated as (SGR % increase in body weight per day) = [(lnWf - lnWi) / t] × 100, where Wf = final weight (g), Wi = initial wet weight (g), and t = time (days). Coefficient of variation (CV) of final fish body weight (BW) was calculated (Kestemont *et al.*, 2003) followed by size heterogeneity = CVwf / CVwi; where wf = final weight, wi = initial wet weight, and CV = coefficient of variation (100 SD / mean).

Statistical analysis

A one-way ANOVA was used to compare the means among treatments of survival, (previously transformed arcsin square root), growth in weight among treatments per week of bioassay (g), weight growth per treatment among bioassay weeks (g) initial and final length (cm), initial and final wet weight (g), coefficient of variation (fish body weight g), size heterogeneity (fish body weight g), Fulton's K (K) and SGR (% d^{-1}). A significance level of $P < 0.05$ was used. Levene's test and residual plots were used to test homogeneity of variance, and the Kolmogorov-Smirnov test was used to corroborate normality. Tukey's HSD *post-hoc* test was used to identify differences among treatment means (SPSS 17.0).

RESULTS

There were no significant differences in either juvenile length ($F_{2,9}$ =0.957, P = 0.436) or wet weight ($F_{2,9}$ = 1.309, P = 0.337) among treatments at the start of the trial. After eight weeks no significant differences were found between treatments in survival ($F_{2,9}$ = 0.700, P = 0.533) coefficient of variation ($F_{2,9}$ = 0.210, P = 0.817), in size heterogeneity ($F_{2,9}$ = 0.006, P = 0.994) and Fulton´s K ($F_{2,9}$ = 0.420, P = 0.675) (Table 1). Significant differences were found among treatments in final length ($F_{2,9}$ = 8.724, P =0.017), final wet weight ($F_{2,9}$ = 11.399, P = 0.009) and specific growth rate ($F_{2,9}$ = 7.080, P = 0.026), where D4 was significantly greater than D12 but similar to D8 and D8 similar to D12 in the three growth parameters (Table 1). The growth in weight during the time of the experiment was statistically similar between treatments until the second intermediate weight measuring, from the third among the treatments (21 days) a significant difference in

growth was observed among the treatments D4, D8, and D12, similar to the final growth data (Fig. 1). In treatments D4 and D8, there were significant differences among the weeks of the experiment within each treatment, while the D12 treatment between the last two weeks there was no significant difference in weight (Fig. 1). From day 20, the three treatments' growth trend began to differentiate; the tendency expressed by growth curves (y = b × e^{ax}) with correlation coefficients of 99% indicates that although there are no significant differences between treatments D4 and D8 in their final weight. At 58 days, D4 treatment would reach 25 g while D8 would have 20 g and D12 18 g. D8 and D12 would reach 25 g in 7 more days (day 65) and 12 more days (day 70), respectively (Fig. 2).

DISCUSSION

Greater growth (weight and length) of fish cultured in D4, compared to the fish cultured in D12, could be explained by an increase in food consumption and nutrient assimilation in the D4 due to the decrease of competition. Although aggressive behavior was observed during feeding, intraspecific competition cannot be considered the only factor that allowed for that difference to occur. The absence of differences in the growth of the fish cultured in D8 with the rest of the treatments may suggest that the species' optimal stocking density lies near these levels.

During the first 20 days, there were no growth differences in weight (6 g) and survival (100%) between treatments, suggesting the use of high density as a management strategy for this species juveniles, in ornamental and food production systems based on profitability. Where high densities of organisms could be maintained up to this weight and then make density adjustments according to the purpose of production, low densities (100 ind m^{-3}) for ornamental and intermediate systems for food production systems (200-250 ind m^{-3}), from the point of view of profitability the D8 treatment, could offer a balance between the cost-benefit per organism per cubic meter over. Similar to this behavior, Mendoza-Alfaro *et al.* (2010) reported in *Atractosteus spatula* that in the first 10 days of age, this fish does not show growth problems or cannibalism.

In regards to the present study, the highest fish growth in D4, when compared to D12, could be related to the decrease in stress-related factors from the generalized intraspecific competition in each tank during feeding in D4, which coincides with McCarthy *et al.* (1999) and Wong & Benzie (2003), who reported that low competition increases food availability reflected in higher growth and greater SGR (Wong &

Table 1. Effect of three stocking densities in wet weight and length (initial and final), wet weight and length gain, survival, specific growth rate (SGR), size heterogeneity, Fulton's K and coefficient of variation (mean ± 1 standard deviation of three replicates per treatment) in *Atractosteus tropicus* cultured for eight weeks. Three (D3), six (D6) and nine (D9) juveniles per each 40 L tank. Means with different superscripts within a row are significantly different (one-way analysis of variance, *P* < 0.05).

| Stocking density (ind)	4	8	12
Final observed survival (%)	100.00 ± 0[a]	91.66 ± 14.43[a]	97.22 ± 4.81[a]
Initial mean weight (g)	3.88 ± 0.14[a]	3.73 ± 0.06[a]	3.81 ± 0.12[a]
Final mean weight (g)	21.41 ± 1.50[a]	18.04 ± 0.93[ab]	16.16 ± 1.56[b]
Weight gain (g d^{-1})	0.2337 ± 0.0216	0.1908 ± 0.01290	0.1650 ± 0.0205
Coefficient of variation (final body weight g)	23.00 ± 6.52[a]	25.96 ± 6.40[a]	25.13 ± 4.11[a]
Size heterogeneity (body weight g)	0.99 ± 0.33[a]	1.01 ± 0.28[a]	0.94 ± 0.20[a]
Initial mean length (cm)	9.25 ± 0.25[a]	9.12 ± 0.12[a]	9.08 ± 0.04[a]
Final mean length (cm)	15.54 ± 0.19[a]	14.79 ± 0.25[ab]	14.23 ± 0.58[b]
Length gain cm d^{-1}	0.0839 ± 0.0048	0.0756 ± 0.0021	0.0687 ± 0.0081
Fulton's K	0.56 ± 0.01[a]	0.55 ± 0.01[a]	0.55 ± 0.01[a]
SGR (% d^{-1})	2.27 ± 0.13[a]	2.09 ± 0.08[ab]	1.93 ± 0.11[b]
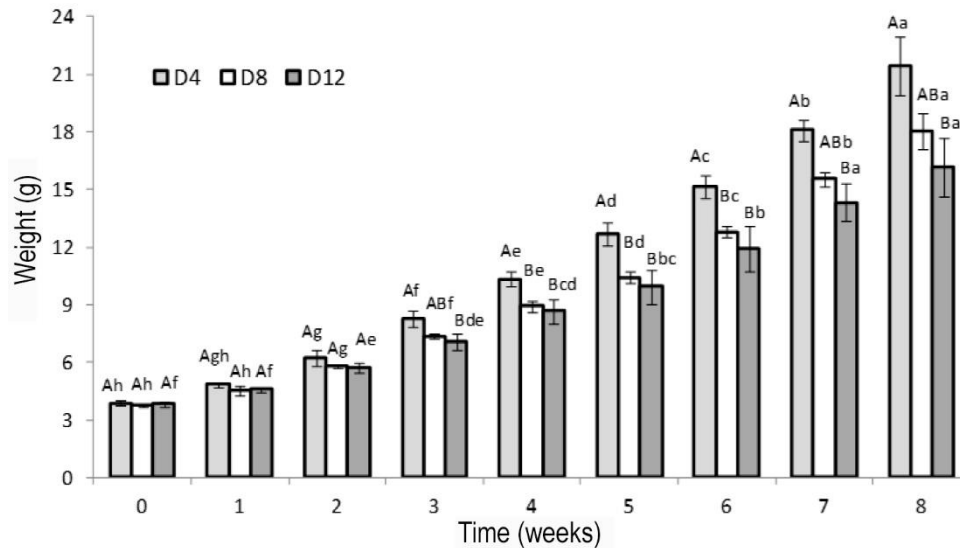
Figure 1. Wet weight of juvenile *Atractosteus tropicus* cultured at three different densities (100, 200 and 300 ind m^{-3}) in a growth trial. Fish were fed at a ratio of 5% body weight per day adjusted daily based on growth and mortality. All values represent the mean of three replicates per treatment ± 1 standard deviation. The different capital letters on the bars show statistical differences among the treatments per week of bioassay and the different lowercase letters on the bars show the statistical differences between weeks per treatment (*P* < 0.05).

Benzie, 2003; Alhassan *et al*., 2012). Although the condition index in the three treatments was similar, this aspect needs further research.

The density of the fish varies according to the stage of growth in which it is; at present, the studies conducted indicate that the larger and heavier the individuals, their density should decrease to preserve optimal growth and avoid problems due to competition. Considering juvenile fish (0.5 to 150 g) with approximately 21 to 270 days after hatching (Márquez-

Couturier & Vázquez-Navarrete, 2015), densities used with this genus have been reported during early stages from 300 ind m^{-3} on *Atractosteus tropicus* (Martínez-Cárdenas *et al*., 2018), to 500 ind m^{-3} on *A. spatula* (Clay, 2009), with a decrease to 20 ind m^{-3} on *A. tropicus* of 100 g, and reaching 10 ind m^{-3} as they approach 400 g (Palma-Cancino *et al*., 2019). Perschbacher (2011) reported in *A. spatula* densities in adults (200 to 450 g) of 3 to 1.5 ind m^{-3}. Martínez-Cárdenas *et al*. (2018) on *A. tropicus* after eight weeks
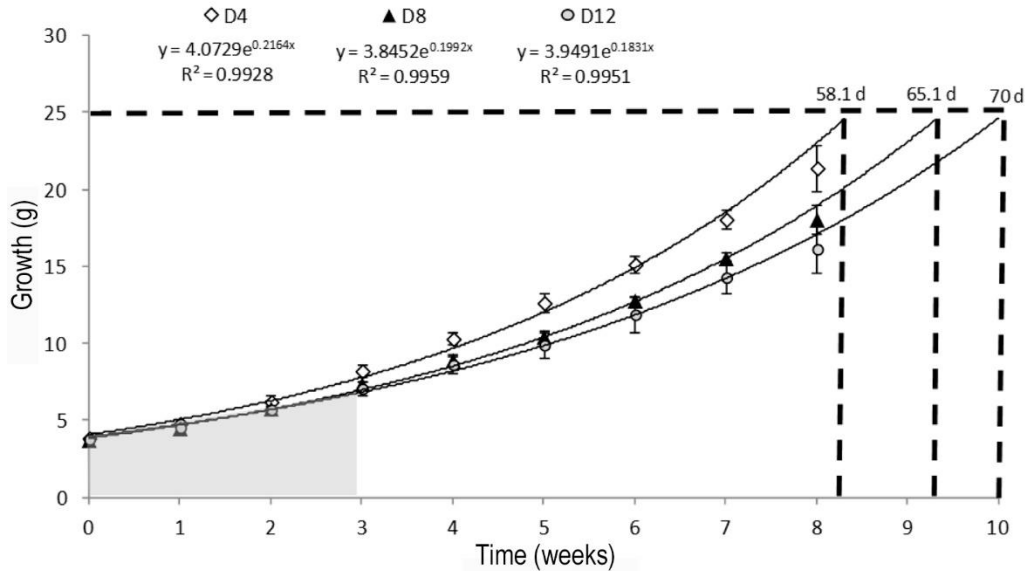
Figure 2. Growth trend of juveniles of *Atractosteus tropicus* at three different densities (100, 200 and 300 ind m^{-3}). The vertical lines on each point show the standard deviation of the data. Continuous lines show the trend of exponential growth of treatments (W = b × L^{ax}). Dotted lines show the projection of growth at 25 g of the three treatments. The shaded area under the graph shows the similarity time of weights among treatments.

of culture with 300 ind m^{-3} reported growth and an SGR similar to the maximum density evaluated in this study without adverse effects, similar to Mendoza-Alfaro *et al.* (2010) on *A. spatula*, who reported a growth of 3.18 mg d^{-1} for juveniles, resulting in a weight gain of approximately 22 g for fish with an initial weight of 5 g.

Present study results coincide with those reported by M'balaka *et al.* (2012), who evaluated three different densities (5, 7 and 9 ind m^{-3}) for 10 weeks in three strains of tilapia *Oreochromis shiranus* with an initial weight of 6.7 g. The authors did not find significant differences between the two lowest densities; poor growth performance was recorded at the highest density, choosing as best density 5 ind m^{-3}. Similarly, Chakraborty & Banerjee (2010) in Nile tilapia (*Oreochromis niloticus*) production systems tested densities of 0.5, 1, 1.5, 2, 2.5 and 3 ind m^{-3}, finding significant differences in growth, with an inverse trend between growth and density and proposed as optimal density 2 ind m^{-3}. Contrary to these results, Osofero *et al.* (2009) evaluated in *O. niloticus* juveniles in culture densities of 50, 100, 150 and 200 ind m^{-3}; the authors found no significant differences among treatments after three months, concluding that the treatment 150 ind m^{-3} was the best, in addition to the growth parameter it was best in cost-benefit between the number of individuals and final biomass produced.

These differences and growth results related to culture densities depend on the life stage, species, and purpose of production. The culture density serves to show the concentration of the fish that are per space unit, gaining relevance for the best use of the system, raw production yield and water utilization, which serves to find maximum utilization of food and space with less stress, minimum energy expenditure and greater growth of fish (Chakraborty & Banerjee, 2010).

The overall absence of significant differences in Fulton's K, size heterogeneity, and coefficient of variation recorded in the present trial, coincides with the results of various studies such as the work on the effect of stocking density on discus, *Symphysodon aequifasciatus* by Tibile *et al.* (2016). However, the authors suggested that the absence of significant differences in condition index and size in the discus was due to limiting sample numbers. Ellis *et al.* (2002), in a review paper, found that there is no apparent effect of stocking density on the condition index of rainbow trout *Oncorhynchus mykiss*; in that study is suggested that stocking density does not have a forceful effect on that species. Riche *et al.* (2013) concluded that stocking density does have a negligible effect in the condition of market size cobia (*Rachycentron canadum*). The poor effect of stocking density on the fish condition was consistent even in a study conducted by Aragón-Flores *et al.* (2014), in which the authors (contrarily to the resulting tendency of the majority of the studies, where the lower the stocking density, the greater the growth) found greater growth and survival on the cichlid *Cichlasoma beani* cultured in relatively high densities.

From the results of the present study, it can be concluded that under the experimental conditions described, the low mortality rates, overall good condition and the similar growth of *A. tropicus* cultured in D8 compared with that recorded on the fish in D4 could be considered an attribute for commercial aquaculture, as it seeks to optimize the use of infrastructure by culturing fish at high stocking densities at least to a particular stage of the life cycle without showing adverse effects. However, future studies are needed to assess the optimal stocking density to improve growth and condition in this species.

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