Research Article

Effect of feeding frequency on growth and survival in juvenile gar *Atractosteus tropicus* Gill, 1863, in culture conditions

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ABSTRACT. The gar, *Atractosteus tropicus* is a native fish distributed in fluvial systems of the Mexican southeast, which presents aquaculture potential in the food industry and as ornamental species. However, it is necessary to deepen the knowledge for the optimization of its culture. The present study examined the effect of three feeding frequencies on the growth, survival, and condition of juveniles. The fish (90) were placed in nine 35 L tanks, (n = 10; three replicates per treatment). The feed (44% protein, 15% fat) was supplied to 2% of the biomass, divided into 2, 4 and 6 servings throughout 10 h for eight weeks. After eight weeks the standard length, wet weight, survival, Fulton K, specific growth rate, nitrogen carbon rate, the coefficient of variation and size heterogeneity were recorded, which were analyzed with a one-way ANOVA. There were no significant differences among treatments for any of the response variables. The overall results indicated that in general both the culture conditions and the three food frequencies tested were adequate for the species. The results indicate the high flexibility of the species to be cultured with minimum requirements of the food supply, which can translate into the optimization of production costs.

Keywords: aquarium, pellets, condition, carbon, nitrogen rate, moisture content, aquaculture.

INTRODUCTION

The gar, *Atractosteus tropicus* is a native tropical fish, distributed in southeastern freshwaters systems in Mexico, where it represents a food source for local communities, although it is present in Nicaragua, Guatemala and Costa Rica and it is considered a living fossil, in the wild feeds mostly on fish and microcrustacean (Miller *et al.*, 2009; Nelson *et al.*, 2016). In Mexico, populations of native fishes are under pressure due to anthropogenic alteration of habitat and introduction of exotic species (Jelks *et al.*, 2008). At present, *A. tropicus* is reproduced in commercial farms (Alvarez-González *et al.*, 2007) following techniques based on studies of its life cycle in captivity through controlled reproduction using hormonal inducers and reports on early stage (juvenile) nutritional requirements. The species diets used in commercial farms are not species specific as the larval stage is fed with *Artemia*, and commercial trout food is used in early juveniles. *A. tropicus* is a species with potential for the diversification in Mexican aquaculture for food consumption, and currently, it is being marketed as an ornamental fish in the aquarium trade (Márquez-Couturier *et al.*, 2006).

The determination of the optimum number of daily meals, to improve fish performance under culture may depend on the aims of the production, operational lo-

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gistics or technical and infrastructural limitations (Baras *et al.,* 2011). An adequate feeding protocol can optimize food consumption, and nutrient assimilation (Tucker, 1998) minimize feed wastage and water pollution (Puvanendran *et al.,* 2003) and improvement of growth performance and immunity of aquatic animals (Ding *et al.*, 2017). The use of a high feeding frequency was found to be advantageous for higher growth and survival in early stages of fish (Murai & Andrews, 1976; Hancz, 1982; Folkvord & Ottera, 1993; Tian *et al.*, 2015; Aderolu *et al*., 2017), while a sub-optimal ration may result in reduced growth and increased size variation (Tyler & Dunn, 1976). However, these findings were reported for different species, while the response to the number of daily meals may depend on the species and stage on its life cycle.

Several studies have been conducted to establish the administration of the appropriate food for the species of economic importance (Lall & Tibbetts, 2009), and have focused on the assessment of food quality such as the study of Azzaydi *et al*. (1999). However, the knowledge of the optimal ration for any species allows supplying the necessary food to achieve the highest efficiency and obtain the maximum growth of fish as well as reduction of overfeeding (García, 2004; Riche *et al.*, 2004; Vega-Villasante *et al.*, 2011; Lisboa da Cunha *et al*., 2013; Costa-Bomfim *et al*., 2014, Kurt-Kaya & Bilguyen, 2015;). Optimization of feeding strategies improves growth, survival and feed conversion factor, decreases size variation and increases production efficiency. Among these feeding strategies is the feeding frequency, which can vary its optimal depending on the species, age, size, environmental factors, and the quality of food, in particular the levels of proteins and energy (Goddard, 1996; Lee *et al.*, 2000; Al Zahrani *et al*., 2013; Benli *et al*., 2015;). With this in mind, the present study aimed to compare the performance of juvenile *A. tropicus* cultured with three feeding frequencies on growth and survival after an eight weeks trial.

MATERIALS AND METHODS

System design and general methods

A total of 120 juvenile gar (0.25 ± 0.0007 g, 8.96 ± 0.0473 cm; mean ± SE) were allocated to a 200 L holding tank at a temperature of 26°C and salinity of 0 g L^{-1}. Each 35 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 0800 h, lights off 2000 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} sec^{-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 Inc; 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.

Feeding adjustments were calculated based on the daily mortality (assigned the previously recorded mean weight) per tank (the rations corresponding to mortalities were not fed to the remainder of fish). Gar length (distance between the tip of the mouth to the tip of the caudal peduncle) was measured by placing the fish on a 1 mm scaled sheet covered by plastic. Gar wet weight was measured on an electronic balance and recorded to the nearest 0.01g. Weekly growth was also recorded from bulk measures of wet weight. Fish were not fed for 24 h before each weighing.

Effect of feeding portions on the growth and survival of juvenile gar *A. tropicus* in culture conditions

The ninety-gar juvenile used in this experiment were feed 2, 4 and 6 portions per day for an 8-week period. The food provided was pellet (Nutripec 1.5 mm particle, 44% protein, 15% fat) a ration rate of 2% body weight (BW) per day (dry weight pellet: wet fish weight). The fish were allocated into each 35 L tank (ten fish per tank, three treatments with three replicates). After eight weeks, the surviving of gars was counted and their weight and length measured individually. Fulton's K was calculated as $K = (W L^{-3}) \times 100$ where W: wet weight (g) and L: total length (cm). Specific growth rate (SGR) was calculated as (SGR % increase in body weight per day) = $[(\ln W_f - \ln W_i)/t] \times 100$, where W_f: final weight (g), W_i: 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 = CV_{wf} /CV_{wi}; where w_f: final weight, w_i: initial wet weight, and CV: coefficient of variation (100 SD/mean).

Moisture and nitrogen/carbon content

The weight of the gar did not meet the minimum quantity of tissue required to conduct conventional proximate analyses. Instead, analyses were conducted to quantify moisture, nitrogen and carbon content in the carcass to determine if gar metabolized food more efficiently in one of the feeding frequencies tested. At the end of the experiment, one gar per tank (randomly selected) was euthanized with an overdose of benzocaine (400 mg L^{-1}), blotted dry and weight and length recorded. Each whole gar was freeze-dried until constant weight was achieved. Also, as low moisture content has been associated with a good condition in fish (Shackley *et al.*, 1993), those dried samples obtained were used for moisture content by determining the difference from wet weight. The gar was then individually ground with a mortar and pestle for analysis of nitrogen and carbon by oxidation/IR detection, using a CHN Organic Elemental Analyzer Flash 2000 (Thermoscientific®).

Statistical analysis

A one-way ANOVA (SPSS 17.0) was used to compare the means among treatments of: survival, initial length, final length (cm), initial weight, final wet weight (g), coefficient of variation (fish body weight g), size heterogeneity (fish body weight g), moisture (%), C: N ratio, 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 the homogeneity of variance. Normality was tested using a Shapiro-Wilk test. 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 ($F_{2,9} = 0.754$, $P = 0.511$) or wet weight ($F_{2,9} = 0.320$, $P = 0.738$) among treatments at the start of the trial (Table 1). After eight weeks, there were not significant differences in length ($F_{2,9} = 1.492$, $P = 0.298$), wet weight ($F_{2,9} = 3.450$, $P = 0.101$), specific growth rate ($F_{2,9} = 4.254$, $P = 0.071$), survival ($F_{2,9} = 0.600$, $P = 0.579$), coefficient of variation ($F_{2,9} = 1.847$, $P = 0.237$), size heterogeneity ($F_{2,9} = 2.431$, $P = 0.169$), Fulton's K ($F_{2,9} = 2.434$, $P = 0.168$), moisture ($F_{2,9} = 0.325$, $P = 0.734$) or C:N ratio ($F_{2,9} = 0.133$, $P = 0.878$) (Table 1).

DISCUSSION

The lack of significant differences in the present study may find an explanation in the trophic physiology of the species, which is not of fast swimming habits in agreement with its habitat of slow waters and whose predatory strategy consists in sneaking stealthily to its prey and once achieved the intake, remains again in slow swim (Kammerer *et al.*, 2006; Miller *et al.*, 2009; Nelson *et al.*, 2016). The present study was conducted with early juveniles taking into consideration the maximum sizes reached by the species. In the juveniles cultivated in the present study, a behavior very similar to that considered for a late juvenile stage of other fish species was observed. It represents an advantage for the optimization of culture practices since, based on the results, the species does not require to be fed in several portions, since only two portions were sufficient to satisfy their nutritional needs, resulting in reduced labor costs.

In the present work, the feed was provided with 44-45% protein and 15% lipids as indicated for early juveniles of *A. tropicus* (Márquez-Couturier *et al.*, 2006). There were no significant differences in growth rate and weight gain, which confirms that the juveniles of this species can hydrolyze artificial food, due to its high proteolytic capacity (Guerrero-Zárate *et al.*, 2013). The feeding rate for the life stage used in the present study in *A. tropicus* is suggested to be up to 7% of its daily biomass depending on the size reached (Márquez-Couturier *et al.*, 2006). However, in the present study 2% of the daily biomass was sufficient to satisfy its nutritional needs, Which gives another advantage for its culture in comparison with other species such as tilapia (*Oreochromis* spp.), which necessarily requires, despite its accelerated growth, up to 6% of their wet weight in food for its optimal growth in this stage (5-6 g) (Vega-Villasante *et al.*, 2011).

A smaller number of rations implies a smaller loss of food, depositing less amount of organic matter in the bottom of the ponds, which prevents the appearance of pathogenic organisms. The results obtained in the present study, by decreasing the number of food rations, not only demonstrated that fish can grow normally and that a farm could maintain its productive indicators, but would also achieve a saving in food and an environmental benefit to waste less food, contributing to the sustainable cultivation of *A. tropicus*. Although the species is considered an opportunistic carnivorous predator, based on its digestive capacity (Frías-Quintana *et al.*, 2015), in the present study, there were no cases of severe aggression that generated a high mortality rate, compared to other species. When grown at relatively low densities some cichlid species show severe aggression (Aragón-Flores *et al.*, 2014), which may be intensified if individuals are close to a change in the stage of development, such as sexual maturity. In the present study, the selected density was

Table 1. Survival, initial and final wet weight, initial and final length, the coefficient of variation, size heterogeneity, moisture, C: N ratio, Fulton's K, and specific growth rate (SGR) (mean ± 1 SE of three replicates per treatment) of *A. tropicus* cultured with three feeding frequencies in an eight weeks trial. Means with different superscripts within a row are significantly different (one-way ANOVA, $P < 0.05$).

Feeding-frequency (Times)	2	4	6
Final observed survival (%)	100.00 ± 0.00	96.66 ± 3.33	93.33 ± 6.66
Initial individual weight (g)	5.21 ± 0.04	5.25 ± 0.03	5.26 ± 0.07
Final individual weight (g)	23.26 ± 0.84	27.84 ± 1.35	27.36 ± 1.72
Coefficient of variation (final body weight g)	19.48 ± 1.81	22.13 ± 1.67	26.46 ± 3.75
Size heterogeneity (body weight g)	0.93 ± 0.06	1.01 ± 0.11	1.25 ± 0.13
Initial length (cm)	10.68 ± 0.07	10.81 ± 0.01	10.78 ± 0.11
Final length (cm)	15.80 ± 0.20	16.30 ± 0.30	16.31 ± 0.19
Moisture (%)	73.56 ± 0.86	73.66 ± 0.43	73.04 ± 0.28
C:N ratio	3.89 ± 0.07	3.93 ± 0.04	3.93 ± 0.06
Fulton's K	0.59 ± 0.02	0.64 ± 0.01	0.62 ± 0.01
SGR (% d^{-1})	2.67 ± 0.06	2.97 ± 0.08	2.94 ± 0.09

adequate for the experiment; however, experimentation is required to determine the optimum culture density for the species at this stage of the life cycle.

Studies on the feeding frequency on growth have been carried out on several fish species of commercial and ornamental importance, with data contradictory with the present study, found even in the same species at similar life stages. This leads to the development of research where some environmental factors can be correlated with the frequency and quantity of food offered for the optimal growth of organisms and the greater profitability of the system. Similar to the results of the present study there were no significant differences between treatments for growth in *Cirrhinus mrigala*, *Labeo rohita* and *Cattla cattla* fed with one, two and three portions (Biswas *et al.*, 2006a, 2006b). In juvenile bluegill (*Lepomis macrochirus*) and hybrid bluegill (*L. cyanellus* × *L. macrochirus*), there were no differences between feeding regimes in final fish weight, survival, weight loss as a percent of initial weight, condition factor, and SGR for either taxon of fish (Roy *et al*., 2017). For *Dicentrarchus labrax* offering two and three times a day (Güroy *et al.*, 2006) and for *Pseudoplatystoma fasciatum* fed two, four and six times per day (Guerra *et al.*, 2009). Similarly, in *Oreochromis niloticus* García & Villarroel (2009) and Vega-Villasante *et al.* (2011) did not report differences between treatments of high frequency of feeding (up to eight portions per day) and low frequency (two portions per day). Al Zahrani *et al.* (2013) found no difference between camouflage grouper (*Epinephelus polyphekadion*) fingerlings fed 1, 2 and 4 times a day. As well as Costa-Bomfim *et al.* (2014) on *Rachycentron canadum* juveniles fed 1, 2, 3, 4 and 6 times a day. However, some studies have found benefit in the growth of several species when using a specific food frequency, contrary to the results of the present study. Ustaoglu & Alagil (2009) on *Oncorhynchus mykiss* (two and six portions per day), Riche *et al.* (2004b) on *O. niloticus* (two and five portions daily), Benli *et al.* (2015) on *Gobiocypris rarus* (one, two and three portions daily), Xiang-Fei *et al.* (2014) on *Megalobrama amblycephala* (one, two, three, four, five and six portions daily), Farooq-Aga *et al.* (2017) on rohu *Labeo rohita* (one and two portions daily) and Fan *et al.* (2017) on yellow catfish *Pelteobagrus fulvidraco* (one, two, three and four portions daily) coincide when reporting that two portions per day as feed frequency are optimal for the growth of these organisms compared to higher feed frequencies. Riche *et al.* (2004a) emphasize that feeding frequency be related to gastric emptying and return of appetite, suggesting that a lower feed frequency will give a greater use of the food without over-gastric load. Lisboa da Cunha *et al*. (2013) found on *Trachinotus marginatus* fed 2, 6, 8, and 10 times a day; only the weight was significantly lower in fish fed 2 and 6 times a day. In moonfish hybrids (*Lepomis cyanellus* × *Lepomis macrochirus*) (three portions daily) (Wang *et al.,* 1998), in tilapia *Oreochromis niloticus* (≥four portions daily; Sanches & Hayashi, 2001; Kurt-Kaya & Bilguven, 2015), in *Oncorhynchus mykiss* (three portions daily) (Ruohonen *et al.*, 1998) in *Salmo trutta labrax* (three portions daily) (Bascinar *et al.*, 2007) and in juveniles *Megalobrama amblycephala* (three portions daily) (Tian *et al.*, 2015). Aderolu *et al.* (2017) found that satiation feeding, two to three times daily, would enhance growth indices in *C. gariepinus* fingerlings suggesting that the effect of a food frequency is species specific and age specific.

Based on what was found in the present study it can be concluded that for the experimental conditions presented there is no difference between the feeding frequencies for early juveniles of *A. tropicus* and it can be suggested that can be fed twice a day with 2% of their wet weight. These results contribute to improve the technology of early-stage culture of the species and can have an economic impact on their production systems through food optimization.

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