Effect of Stocking Density on Growth, Survival, and Condition of the Mexican Cichlid *Cichlasoma beani*

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Abstract

The Mexican cichlid *Cichlasoma beani* is currently exploited regionally as food and can be commercialized in the aquarium trade. Natural populations of *C. beani* may already be negatively affected by anthropogenic alteration of the areas in which it is distributed. The aim of the present study was to examine the effect on growth, survival, and condition of *C. beani* cultured in three stocking densities: three (D3), six (D6), and nine (D9) fish per each 40 L tank. At the end of a 6-wk trial the fish cultured in D3 were longer, heavier, and grew faster than the rest of the treatments but their survival was the lowest compared to D6 and D9. The mortalities were caused by a strong aggressive behavior in D3.

The Mexican cichlid *Cichlasoma beani* (Cichlidae) is distributed within the Pacific slope (Miller et al. 2005). Natural populations of *C. beani* may already be adversely affected by the introduction of exotic fish species (such as tilapia) and water pollution generated by crop fields adjacent to the areas in which *C. beani* inhabits, which can also alter and fragment aquatic systems (Vitousek et al. 1997). *C. beani* is currently exploited regionally as food and has potential to be part of the aquarium trade as

many aquarists seek relatively unknown species to complement their collections. For that purpose, the culture of *C. beani* may prevent unregulated fishing that can negatively affect natural populations (Waples et al. 2007).

The development of culture techniques has been a successful strategy to protect species (Pérez-Sánchez and Páramo-Delgadillo 2008; Martinez-Cardenas and Purser 2011). Suboptimal stocking densities can cause hierarchical social stress because of territorial and food competition. Moreover, fish can be susceptible to suboptimal food distribution, diseases, and size

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heterogeneity (Auro and Ocampo 1999). The aim of this study was to examine the effect on growth, survival, and condition of *C. beani* cultured in three stocking densities: three (D3), six (D6), and nine (D9) fish per each 40 L tank.

Materials and Methods

Collection and Maintenance

The fish were caught at the site "El Chilte," Tepic, Nayarit, México (21°38′42″N, 104°48′ 26″W). From the site, 70 fish (mean ± 1 SE) 8.84 ± 0.54 cm in standard length (tip of the snout to base of caudal fin) and (mean ± 1SE) 15.9 ± 6.11 g in wet weight, were transported in a plastic tank of 200 L under conditions similar to the collection site, water at a temperature of 26 C and a salinity of 0 g/L. Continuous aeration was provided during transit. The wet laboratory where experiments were conducted was located in Tepic, Nayarit, Mexico. Prior to the experiment, the fish were located in a holding tank at a water temperature of 26 C and a salinity of 0 g/L.

Three separate 160-L recirculation systems were used simultaneously for the experiment; each system had three 40-L tanks connected to a biofilter comprised of a 40-L plastic container. The outflow from the tanks was uniformly released into a rectangular plastic strainer covered with dacron to retain solids and filled with 40-mm bioballs. The filtered water trickled down to the container below. This lower container was used as a water reservoir in which a 40 W submersible pump (Resun, Shenzhen, China) of a 1400 L/h delivery volume was installed. The pump provided an inflow of approximately 156 L/h/tank set with the use of PVC spherical valves. In the reservoirs a 200 W heater (Hagen, Montreal, Canada) was set to maintain 30 C as this level has been used successfully on the species, which inhabits in the wild at water temperatures up to 34 C as observed in certain sites during collection. A 12:12 (L : D) photoperiod was provided (lights on at 0800 h, lights off 2000 h) by a timer controlled cool white light of 35 W (General Electric Company, Fairfield, CT, USA) producing an intensity of 5.2 μE/m^2/sec at the water surface.

Two 10-cm long segments of PVC pipe were placed in each tank to provide shelter for the fish and reduce the aggressive behavior typical of the Cichlidae family (Grant et al. 2002; Leiser et al. 2004; Arnott and Elwood 2009; Heg 2010; Lorenz et al. 2011).

Effect of Three Stocking Densities on Growth, Condition, and Survival of C. beani Cultured for 6 Wk in Three, Six, and Nine Individuals per each 40 L Tank

A total of 54 individuals were placed in nine 40 L tanks for 6 wk. Three culture densities were tested, treatment D3 consisted in a stocking density of three individuals per tank (one fish per 13 L of water), in treatment D6 were located six individuals per tank (one fish per 6.6 L of water), and in treatment D9 were located nine individuals per tank (one fish per every 4.4 L of water). At the beginning of the trial, a broad-spectrum anthelmintic (Praziquantel Microbe-lift, FL, USA) was administered at a dosage of 7 mg/L as a preventive measure against the nematode *Eustrongylides sp*, which caused mortalities in a preliminary trial.

The fish were fed aquarium fish flakes (42% protein, 5% fat) offered at a ration rate of 5% body weight per day (dry weight food : wet weight fish) divided into three equally sized meals (0800, 1200, and 1600 h). 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).

Water quality was maintained as follows: dissolved oxygen > 75% saturation, total ammonia nitrogen (TAN) < 0.5 mg/L, nitrite < 0.25 mg/L, nitrate < 5 mg/L. Average pH was 7.85 (7.6–8.1). For the determination of pH, TAN, nitrite, and nitrate, a colorimetric saltwater liquid test kit (Aquarium Pharmaceuticals, Inc., Chalfont, PA, USA) was used. Temperature was monitored every 24 h, whereas TAN, pH, nitrite, and nitrate were recorded every 48 h. Tanks were inspected daily for mortalities and any excess food and feces were siphoned to waste.

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 prior to each weighing. Standard length and wet weight of individual fish were recorded on day 0. After 6 wk, the surviving fish were counted and their wet weight and standard length measured individually. Fulton's K was calculated as $K = (W/L3) \times 100$, where W = wet weight (g) and L = standard length (cm). Specific growth rate (SGR) was calculated as (SGR% increase in body weight per day) = $[(lnWf - lnWi)/t] \times 100$, 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). Size heterogeneity and CV were compared only between D6 and D9 as the high mortality rate in D3 did not allow calculation of the respective formula. In one replicate of D6 only one fish survived, which killed the other five fish in the tank. That individual was not used for statistical comparisons.

Moisture and Nitrogen/Carbon Content

To compare the metabolism efficiency among treatments (at the end of the experiment) one fish per tank was randomly selected to be euthanized with an overdose of benzocaine (400 mg/L), blotted dry, and its wet weight and standard length were recorded; each whole fish was freeze-dried until constant weight was achieved. Moisture, nitrogen, and carbon content analyses were conducted to determine if the fish metabolized feed differently among treatments (Westernhagen et al. 1998). Euthanized fish were individually ground with a mortar and pestle for analysis of nitrogen and carbon ratio by oxidation/infrared detection, using a CHNS autoanalyzer.

Statistical Analysis

A one-way ANOVA (SPSS 17.0) was used to compare the means among treatments of survival, initial length, final length (mm), initial weight, final wet weight (g), CV (fish body weight g), size heterogeneity (fish body weight g), moisture content (%), C : N ratio, Fulton's K (K), and SGR (%/day). A significance level of $P < 0.05$ was used. Levene's Test and residual plots were used to test homogeneity of variance. 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 ($F_{2,6} = 2.702$, $P = 0.146$) or wet weight ($F_{2,6} = .201$, $P = 0.823$) among treatments at the start of the trial (Table 1). After 6 wk no significant differences were found between treatments in carbon/nitrogen ratio ($F_{2,5} = 1.545$, $P = 0.300$), moisture content ($F_{2,5} = 1.065$, $P = 0.412$), and Fulton's K ($F_{2,5} = 2.025$, $P = 0.227$). Similarly, no significant differences were found between D6 and D9 in size heterogeneity ($F_{1,3} = 0.564$, $P = 0.507$) and CV ($F_{1,3} = 4.589$, $P = 0.122$) (Table 1). Significant differences were found among treatments in final length ($F_{2,5} = 27.928$, $P = 0.002$), final wet weight ($F_{2,5} = 16.561$, $P = 0.006$), SGR ($F_{2,5} = 24.597$, $P = 0.003$), and survival ($F_{2,5} = 237.031$, $P < 0.000$). D3 presented a greater length and wet weight, but also presented the lowest survival compared to the rest of the treatments (Fig. 2). From the second bulk measuring to the end of the trial, the fish in D3 were heavier than those in the rest of the treatments (Fig. 1).

Discussion

The greater length and weight of the fish cultured in D3, compared to that of the fish cultured in D6 and D9, could be explained by an increase in food consumption and assimilation because of the absence of competition in D3. Based on the aggressive behavior observed during feeding in treatments D6 and D9, intraspecific competition may have been the main factor that prevented the fish (22 g, 8.3 cm) in those treatments to grow similar to the fish in D3 (35 g, 9.9 cm). It has been reported that at high-density intraspecific competition increases, which generates social stress

Table 1. *Effect of three stocking densities in wet weight and standard length (initial and final), survival, specific growth rate (SGR), size heterogeneity, Fulton's K, coefficient of variation, moisture, and C : N rate (mean ± 1 SE of three replicates per treatment) in* C. beani *cultured in recirculation systems for 6 wk. Three (D3), six (D6), and nine (D9) juveniles per each 40 L tank.*[1]

Stocking density	D3	D6	D9
Initial length (cm)	6.73 ± 0.03^a	6.53 ± 0.06^a	6.63 ± 0.03^a
Initial weight (g)	9.30 ± 0.68^a	9.53 ± 0.29^a	9.13 ± 0.23^a
Final length (cm)	9.93 ± 0.24^a	8.25 ± 0.04^b	8.36 ± 0.12^b
Final weight (g)	35.66 ± 2.72^a	22.00 ± 0.99^b	22.03 ± 1.00^b
Specific growth rate (% d)	3.20 ± 0.18^a	2.00 ± 0.01^b	2.09 ± 0.08^b
Coefficient of variation (final body weight [g])	[2]	41 ± 2.44^a	29.01 ± 4.10^a
Size heterogeneity (body weight [g])	[2]	1.31 ± 0.41^a	1.08 ± 0.02^a
Final observed survival (%)	33.33 ± 0.00^a	100.00 ± 0.00^b	96.29 ± 3.70^b
Fulton's K	3.62 ± 0.12^a	3.91 ± 0.10^a	3.75 ± 0.00^a
C : N ratio	4.13 ± 0.09^a	3.91 ± 0.44^a	3.65 ± 0.13^a
Moisture (%)	65.14 ± 2.51^a	68.39 ± 1.77^a	68.35 ± 0.46^a

[1] Means with different superscripts within a row are significantly different (one-way ANOVA, $P < 0.05$).
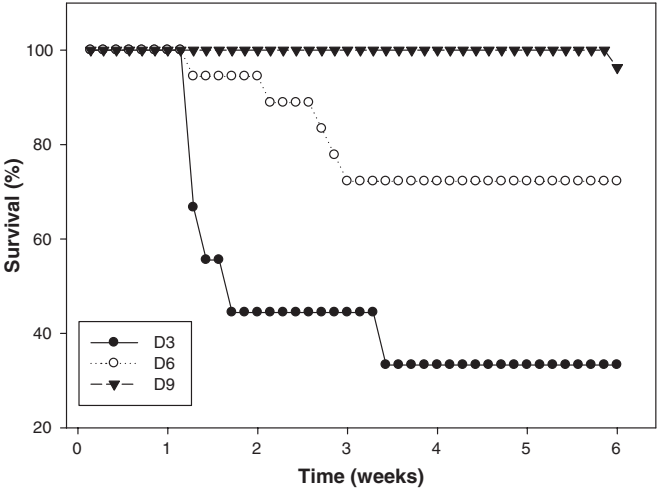
[2] No data because of mortality in D3.

Figure 1. *Daily survival (percent mean of three replicates per treatment) of* C. beani *cultured at three different stocking densities in a growth trial. Fish were fed at a ratio of 5% body weight per day adjusted daily based on growth and mortality. SE bars were omitted to aid visualization.*

that results in poor condition of the attacked fish (Barcellos et al. 1999; Grant et al. 2002). However, in this study the condition in D6 and D9 was not different to that in treatment D3. The overall results of the carbon : nitrogen ratio (all >3) indicated that the fish were not nutritionally stressed in any of the treatments (Harris et al. 1986). The lack of significant differences in Fulton's K and moisture content (all over 65%) suggests that condition in the three treatments was similar and within a normal range for healthy fish. These results robust the possibility that the greatest growth of the surviving fish in D3 could be related to the lack of competition after the surviving (dominant) fish killed the other two individuals in that treatment, which eradicated any stress related to intraspecific competition. This coincides with McCarthy et al. (1999) and Wong and Benzie (2003) who reported that low competition increases the availability of food, which is reflected in higher growth and greater SGR (Jiménez-Martínez et al. 2009; Alhassan et al. 2012).
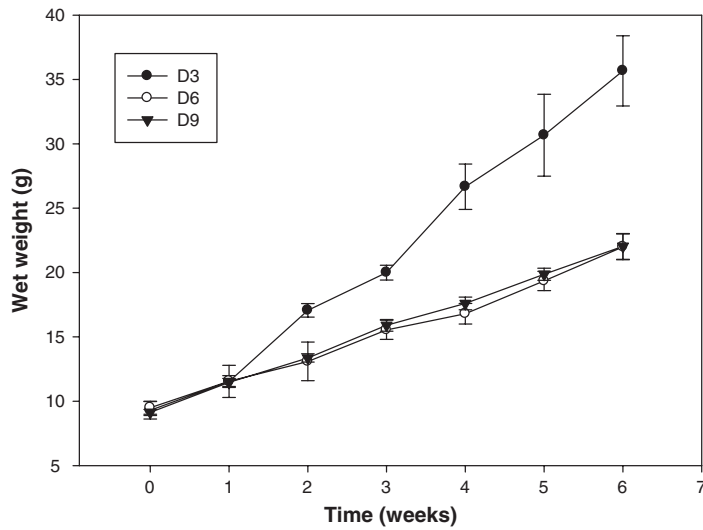
FIGURE 2.   *Wet weight of juvenile* C. beani *cultured at three different stocking densities in a growth trial. Fish were fed at a ratio of 5% body weight per day adjusted daily based on growth and mortality. All values represent the mean of three replicates per treatment ± 1 SE.*

Aggression is common in cichlids (Grant et al. 2002; Leiser et al. 2004; Arnott and Elwood 2009; Heg 2010; Lorenz et al. 2011) and generally occurs in high densities where competition for food and space promote aggressive responses (Rose et al. 2001; Jiménez-Martínez et al. 2009). However, during the present study aggression that caused high mortality rates was observed in the treatment with the lowest density (D3). According to Grant et al. (2002), aggression can be originated by a social hierarchy even at low stocking densities, in some cases because of size variation between individuals (Shubha and Reddi 2011). Unfortunately, owing to the high mortality rate in D3 in the present study, size heterogeneity could not be statistically compared to that in D6 and D9, which did not show significant differences between those treatments. While mean survival rate in D6 and D9 was high (>90%), the survival of only one fish in one replicate of D6 demonstrates that even at that stocking density, a high mortality rate similar to that observed in D3 can take place. The surviving individual was not considered for statistical comparisons because of its size (36 g) relative to the average of the other two replicates of D6 (22 g), where increased growth was most likely a result of reduced competition.

Along with the parasite-related mortality in a preliminary trial (that caused its termination), reproduction in one replicate of D3 was unexpectedly observed (the experimental design was the same as in the fully conducted trial in this study). Fry was observed approximately 2 wk after a "nest" was constructed, but eggs were not detected during this period. The absence of eggs from the nest could be attributed to parental care, as some cichlids carry eggs in their mouth to protect the eggs (Bhujel 2000; Specker and Kishida 2000; Taborsky and Foerster 2004; Watanabe et al. 2006; Reardon and Chapman 2010). However, future studies are needed to verify this strategy in the species. The occurrence of reproduction in individuals cultured in the preliminary trial (weight 21 and 23 g and length 7.5 and 9.5 for female and male, respectively) differs from García-Lizárraga et al. (2011), who reported minimal reproductive size in the species as 10.5 cm (standard length). However, the findings of García-Lizárraga et al. (2011) were obtained from *in situ* data.

In the preliminary trial reproduction was observed after the "third" individual in that replicate was killed because of the constant aggression inflicted by the dominant fish, which was possibly related to reproductive competition

(Lehtonen and Lindstrom 2008). Once the fry was approximately 2 wk the male constantly attacked the female, causing its death by intraspecific competition, which, after completion of the reproductive ritual, was reintroduced as the major cause of aggressiveness between individuals of a couple (Arnott and Elwood 2009). The aggressive behavior observed in the preliminary trial was consistent with the results of the fully conducted experiment, in which the lowest stocking density presented the highest aggressiveness-related mortalities. From the results of this study it can be concluded that under the experimental conditions described, the use of a low stocking density triggered an aggressive response in *C. beani* that led to high mortality rates. The low mortality rates and good condition of the fish cultured at high stocking densities could be considered an attribute for commercial aquaculture, as it seeks to optimize the use of infrastructure by culturing fish at high stocking densities. However, future studies are needed to assess the optimal stocking density to avoid mortalities and improve growth and condition in the species.

Acknowledgements

Literature Cited

Alhassan, E. H., E. D. Albarike, and C. L. Ayisi. 2012. Effects of stocking density on the growth and survival of *Oreochromis niloticus* cultured in hapas in a concrete tank. African Journal of Agricultural Research 7:2405–2411.

Arnott, G. and R. W. Elwood. 2009. Gender differences in aggressive behaviour in convict cichlids. Animal Behaviour 78:1221–1227.

Auro, O. A. and C. L. Ocampo. 1999. Diagnostico de estrés en peces. Veterinaria Mexico 30:337–344.

Barcellos, L. J. G., S. Nicolaiewsky, M. G. De Souza, and L. F. 1999. The effects of stocking density and social interaction on acute stress response in Nile

tilapia *Oreochromis niloticus* (L.) fingerlings. Aquaculture Research 30:887–892.

Bhujel, R. C. 2000. A review of strategies for the management of Nile tilapia (*Oreochromis niloticus*) broodfish in seed production systems, especially hapa-based systems. Aquaculture 181:37–59.

García-Lizárraga, M. A., F. E. Soto-Franco, R. J. M. J. Velazco-Arce, J. I. Velazques-Abunader, J. S. Ramirez-Pérez, and E. Peña-Messina. 2011. Population structure and reproductive behavior of Sinaloa *Cichlasoma beani* (Jordan, 1889) in a tropical reservoir. Neotropical Ichthyology 9:593–599.

Grant, J. W. A., I. L. Girard, C. Breau, and L. K. Weir. 2002. Influence of food abundance on competitive aggression in juvenile convict cichlids. Animal Behaviour 63:323–330.

Harris, R. K., T. Nishiyama, and A. J. Paul. 1986. Carbon, nitrogen and caloric content of eggs, larvae, and juveniles of the walleye pollock, *Theragra chalcogramma*. Journal of Fish Biology 29:87–98.

Heg, D. 2010. Status-dependent and strategic growth adjustments in female cooperative cichlids. Behavioral Ecology and Sociobiology 64:1309–1316.

Jiménez-Martínez, L. D., C. A. Alvarez-Gonzáles, W. M. Contreras-Sánchez, G. Márquez-Couturier, L. Arias-Rodriguez, and J. A. Almeida-Madrigal. 2009. Evaluation of larval growth and survival in Mexican mojarra, *Cichlasoma urophthalmus*, and bay snook, *Petenia splendida*, under different initial stocking densities. Journal of the World Aquaculture Society 40(6):753–761.

Kestemont, P., S. Jourdan, M. Houbart, C. Melard, M. Paspatis, P. Fontaine, A. Cuvier, M. Kentouri, and E. Baras. 2003. Size heterogeneity, cannibalism and competition in cultured predatory fish larvae: biotic and abiotic influences. Aquaculture 227:333–356.

Lehtonen, T. K. and K. Lindstrom. 2008. Density-dependent sexual selection in the monogamous fish *Archocentrus nigrofasciatus*. Oikos 117:867–874.

Leiser, J. K., J. L. Gagliardi, and M. Itzkowitz. 2004. Does size matter? Assessment and fighting in small and large size-matched pairs of adult male convict cichlids. Journal of Fish Biology 64:1339–1350.

Lorenz, O. T., M. T. O´Connell, and P. J. Schofield. 2011. Aggressive interactions between the invasive Rio Grande cichlid (*Herichthys cyanoguttatus*) and native bluegills (*Lepomis macrochirus*), with notes on redspotted sunfish (*Lepomis miniatus*). Journal of Ethology 29:39–46.

Martinez-Cardenas, L. and G. J. Purser. 2011. Effect of temperature on growth and survival in cultured early juvenile pot-bellied seahorses, *Hippocampus abdominalis*. Journal of the World Aquaculture Society 42:854–862.

McCarthy, I. D., D. J. Gair, and D. F. Houlihan. 1999. Feeding rank and dominance in *Tilapia rendalli* under defensible and indefensible patterns of food distribution. Journal of Fish Biology 55:854–867.

Miller, R., W. L. Minckley, and M. S. Norris. 2005. Freshwater fishes of Mexico. University of Chicago Press, Chicago, Illinois, USA.

Pérez-Sánchez, E. and S. Páramo-Delgadillo. 2008. The culture of cichlids of southeastern Mexico. Aquaculture Research 39:777–783.

Reardon, E. E. and L. Chapman. 2010. Hypoxia and energetics of mouth brooding: is a parental care a costly affair? Comparative Biochemistry and Physiology 156:400–406.

Rose, K. A., J. H. Cowan Jr., K. O. Winemiller, R. A. Myers, and R. Hilborn. 2001. Compensatory density dependence in fish populations: importance, controversy, understanding and prognosis. Fish and Fisheries 2:293–327.

Shubha, M. and S. R. Reddi. 2011. Effect of stocking density on growth, maturity, fecundity, reproductive behaviour and fry production in the mouth brooding cichlid *Oreochromis mossambicus* (Peters). African Journal of Biotechnology 10:9922–9930.

Specker, J. L. and M. Kishida. 2000. Mouthbrooding in the black-chinned tilapia *Sarotherodon melanotheron* (Pisces: Cichlidae): the presence of eggs reduces androgen and estradiol levels during paternal and maternal parental behavior. Hormones and Behavior 38:44–51.

Taborsky, B. and K. Foerster. 2004. Female mouthbrooders adjust incubation duration to perceived risk of predation. Animal Behaviour 68:1275–1281.

Vitousek, P. M., H. A. Mooney, J. Lubchenco, and J. M. Melillo. 1997. Human domination of earth's ecosystems. Science 277:494–499.

Waples, R. S., R. W. Zabel, M. D. Scheuerell, and B. L. Sanderson. 2007. Evolutionary responses by native species to major anthropogenic changes to their ecosystems: Pacific salmon in the Columbia River hydropower system. Molecular Ecology 17:84–96.

Watanabe, O. W., T. M. Losordo, K. Fitzsimmons, and F. Hanley. 2006. Tilapia production systems in the Americas: technological advanced, trends and challenges. Reviews in Fisheries Science 10:465–498.

Westernhagen, H., C. Freitas, G. Fuerstenberg, and J. Willfuehr-Nast. 1998. C/N data as an indicator of condition in marine fish larvae. Archive of Fishery and Marine Research 46:165–179.

Wong, J. M. and J. A. Benzie. 2003. The effects of temperature, *Artemia* enrichment, stocking density and light on the growth of juvenile seahorses, *Hippocampus whitei* (Bleeker, 1885), from Australia. Aquaculture 228:107–121.