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Effect of different stocking densities in juveniles of American crocodile (*Crocodylus acutus*, Crocodilia, Crocodylidae) in captivity

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ABSTRACT

Objective: To evaluate the effect of different stocking densities:12 (D12), 18 (D18) and 24 (D24) individuals m^{-2} (ind m^{-2}) on growth (weight and length) and survival in juvenile river crocodiles (*Crocodylus acutus*).
Design/methodology/approach: The crocodiles were cultured in each treatment by triplicate for 43-d in nine plastic tanks and fed a diet based on a mix of beef liver, fish and commercial dog food.
Results: At the end of the experiment, there were not significant differences among treatments in the growth variables, recording a low increment in weight and total length (38.99 ± 8.96 g and 4.19 ± 1.36 cm; mean $\pm$ SD) in all densities as well as overall survival of $62.02\pm7.67\%$ (mean $\pm$ SD). The stocking density of 24 ind m^{-2} was significantly higher in biomass production by m^2 for the stocking densities of 18 and 12 individuals ($p<0.05$).
Limitations on study/implications: The development of culture techniques is a tool to assess the potential of this ecologically important species for its conservation and eventual commercialization.
Findings/conclusions: It can be recommended a stocking density of 24 ind m^{-2} (D24) to optimize the use of space and infrastructure profitability.

Keywords: diet, experiment, juveniles, growth, husbandry, survival.

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INTRODUCTION

The American crocodile (*Crocodylus acutus*) is distributed from Mexico to Peru on the Pacific coast, and from southern Florida to Venezuela in the Atlantic coastal areas (Pérez *et al.*, 2009; Thorbjarnarson, 2010). It is a species threatened mainly by illegal hunting, but also by the destruction and contamination of the ecosystems where they inhabit (Pérez *et al.*, 2009; García-Grajales *et al.*, 2012). In Mexico, this species is categorized as "a species subject to special protection" (SEMARNAT, 2010). Crocodile farming arose because of the demand for skins and meat, and in early stages of their life cycle to be sold as pets (Nickum *et al.*, 2017) due to its high farming potential (Pérez *et al.*, 2009; Grobler, 2012; Isberg and Shilton, 2013; Blessing *et al.*, 2014; Brien, 2015; Brien *et al.*, 2016; Nickum *et al.*, 2017). Crocodile farming has been reported to alleviate the negative effect of overfishing on wild populations, as it provides a steady stream of legally sourced commercial products such as skin, which reduces poaching (Nickum *et al.*, 2017). It also enables the repopulation of the species in the natural environment and reduces the risk of extinction (Pérez *et al.*, 2009; Barrios-Quiroz and Casas-Andreu, 2010).

The research that focused on crocodilian cultivation aspects is useful, so that in addition to their conservation these animals can be exploited in Mexico through the Management Units for the Conservation of Wildlife (WMUs), which provide economic benefits to rural areas by generating sources of employment and economic resources for the use of its inhabitants (Retana-Guiascón *et al.*, 2011; Álvarez-Peredo *et al.*, 2018). They contribute to the conservation of protected species by promoting their breeding and the legal use of their resources within protected areas, thus creating a sustainable balance (Gallina and Escobedo-Morales, 2009). Among the advantages of WMUs are increasing the population of key species or species in some risk category, since they can function as centers for the conservation of genetic material.

Understanding the role of stocking density in a species' behavior is important because each species can show a positive, negative or neutral effect to this condition of captivity by the increase in aggression, which leads to a loss of the quality of the specimens for marketing. Thus, optimizing production by optimizing stocking density will allow better growth and survival (Brien *et al.*, 2016). Most of the studies related to crocodilian cultivation have been carried out on newborns, with a stocking density between 10 and 15 individuals m^{-2} (ind m^{-2}) (Meraz *et al.*, 2008; Barrios-Quiroz and Casas-Andreu, 2010; Bagatto *et al.*, 2012; Brien *et al.*, 2016; Nickum *et al.*, 2017; Isberg *et al.*, 2018), where is it reported 1.9 g d^{-1} growth and survival around 80%. However, this may vary depending on the species, stage, and husbandry conditions (Brien *et al.*, 2016). On the other hand, stocking densities of less than 10 ind m^{-2} present mortality around 70% and an increase in growth of up to 3.71 g d^{-1} (Pérez *et al.*, 2009; Blessing *et al.*, 2014). Poletta *et al.* (2008), Brien *et al.* (2016) and Ciocan *et al.* (2018) tested stocking densities higher than 15 ind m^{-2}, and recorded growth of 1.66 g d^{-1} in early stages of *Crocodylus porosus* and *Caiman latirostris*, respectively, in newborns of up to 1 year of age.

Compared to other aquatic reptiles such as turtles, some studies reported that low densities increase growth. These studies have been reported in the soft-shelled turtle (*Pelodiscus sinensis*) in China, where stocking densities ranging from 3 to 10 ind m^{-2} have better growth performance compared with 12 to 96 ind m^{-2} (Chen *et al.*, 2007; Jing and Niu, 2008; Zhao *et al.*, 2019). However, there have been cases where this trend is not consistent, as in the marine species *Chelonia mydas* (Kanghae *et al.*, 2016). The objective of the present study was to determine the effect of different stocking densities (12, 18, and 24 ind m^{-2}) on the growth and survival in juveniles of *C. acutus* maintained in captivity.

MATERIALS AND METHODS

The present study was conducted with a total of 135 one-month-old individuals (101.86±3.04 g; 32.55±0.14 cm; mean ± SD) of *C. acutus*. The crocodiles used in this study were born at the River Crocodile Rescue and Conservation Center "KIEKARI" (Registry PIMVS: DGVS-PIMVS-IN-1043-NAY/08), located in the Ejido "La Palma" municipality of San Blas, Nayarit, Mexico (21° 32' 25.17" N; 105° 13' 15.19" W). The experiment lasted for 43 d and was carried out from October to November 2017 within the facilities of the "KIEKARI" Center. All applicable international, national and/or institutional guidelines for the care and use of animals were followed by the authors. The juveniles were kept into a conditioned space with a temperature of 30±0.3 °C. Nine plastic tanks of 250 L capacity and 0.25 m^2 were used for the experiment. These tanks were filled to a depth of 5 cm with chlorine-free fresh water. Each tank had a dry area using a wooden platform (0.3 m^2), and a net was placed on top of each tank to prevent escaping.

During the experiment, the crocodiles were fed twice a week. The composition and protein sources of the diet were: fresh beef liver (20%), fresh ground whole fish (65%), and commercial dog food (15%). The ingredients were crushed and mixed in a meat grinder (SANITARY®, model 1275) until a uniform paste was obtained. The proximal composition of the mix was 68% protein and 4.63% fat.

Crocodiles were fed at 15% of their wet biomass. The calculation of the feeding percentage, was adjusted according to mortality and fortnightly growth. The food was placed on the 0.3 m^2 wooden platform to avoid disintegration, leaching, and contamination of the tank water. The food was offered for 2 h, after which the uneaten food was removed and weighed. Cleaning of the tanks and dry areas was carried out weekly, together with a total replacement of the water, and the crocodiles were cleaned with running water. A total of 135 individuals were placed in nine 250 L tanks for 43 d. Three stocking densities (12, 18, and 24 crocodiles m^{-2}; 10, 15, and 20 crocodiles per tank, respectively) were tested in triplicate. The response variables recorded were: 1) survival (%)=100−(# of individuals initial−of individuals final/# of individuals initial)*100; 2) size change (cm d^{-1})=final length−initial length/days of the experiment; weight change (g d^{-1})=final weight−initial weight/days of the experiment; 3) specific growth rate (SGR)=[(ln final weight−ln initial weight)/days of experiment]*100; food consumption (FC)=(food supplied−unconsumed food)/individuals in the tank; 4) food conversion factor (FCF)=food consumed/increase in weight; initial and final stocking density (crocodiles m^{-2}); 5) percentage change in stocking density (%)=initial stocking density−final stocking density; and 6) final biomass produced by m^2 (g m^{-2})=final stocking density *(initial weight per individual−final weight per individual).

The total length and wet weight of each individual of the total population of crocodiles was registered at the beginning of the experiment, and every 15 d following. The total length was calculated from the tip of the tail to the tip of the mouth using an ichthyometer (accuracy 1 mm). The wet weight was recorded with an electronic scale with a precision of 1 g. Additionally, the replica's total biomass per treatment and survival were recorded at the end of the experiment with a hook scale (precision of 1 g), where the individuals were placed on a weighing net. The total biomass was divided by the number of organisms to obtain the average weight per individual per replicate and treatment.

The Kolmogorov-Smirnov test was used for normality (α=0.05) and the Bartlett test to determine homoscedasticity (α=0.05). The variables in percentage rates (survival and stocking density change) were transformed with the arcsine function to its square root prior to its analysis. A one-way analysis of variance (ANOVA) was used for survival, initial stocking density, final stocking density, percentage of stocking density change, biomass/m^2, initial weight, final weight, weight change per day, SGR, initial length, final length, length change, food consumption and FCF. We used a Tukey's multiple mean comparison test to identify differences among treatments ($p<0.05$). All tests were performed through the statistical program SPSS Statistics for Windows, version x.0 (SPSS Inc., Chicago, Ill., USA; IBM Inc., 2020).

RESULTS

At the end of the 43 d of experimentation, not significant differences were observed among the treatments (mean ± SD) for survival (62.04±7.67%), initial and final weight (101.86±2.19 and 140.86±8.75 g, respectively; Figure 1), weight change (0.91±0.21 g ind^{-1} d^{-1}), SGR (0.75±0.15), start and end length (32.55±0.14 and 36.74±1.37 cm, respectively), length change (0.10±0.03 cm ind^{-1} d^{-1}), food consumption (11.36±0.87 g ind^{-1} d^{-1}) and food conversion factor (13.03±2.61) ($p>0.05$; Table 1). The final stocking density of crocodiles had significant differences with a higher value in D24 (15.60±2.40 ind m^{-2}) compared to D18 and D12, which were statistically similar (10.40±1.39 and 7.60±0.69 ind m^{-2}, respectively; $p<0.05$). The final biomass generated per square meter was significantly different among treatments ($p < 0.05$) with 578.52±156.21 g m^{-2} in D24, similar to D18 (421.11±87.72 g m^{-2}) and different to D12 (291.36±59.13 g m^{-2}), which was similar to D18. At the end of the bioassay, the three treatments presented an average weight gain of 38.99±8.96 g, while the length had an increase of 4.19±1.36 cm.

DISCUSSION

There were no significant differences in crocodile survival by treatment, indicating that the stocking densities tested did not influence this factor in the present study. The overall survival of 62% obtained differs from that reported by other studies (Brien *et al.*, 2014; Brien *et al.*, 2016), who reported 28 and 36% survival in early stages of *C. porosus* cultured in captivity, evaluating the effect of stocking density on growth. Contrarily, Meraz *et al.* (2008), recorded 74% survival in their American crocodile growth monitoring study of *C. acutus* during his first year of life in captive conditions.

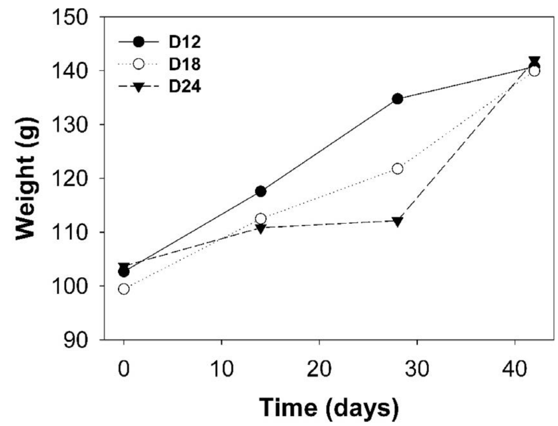

Figure 1. Wet weight (mean ± SD of three replicates per treatment) of juveniles of American crocodile (*Crocodylus acutus*) cultured in three stocking densities 12, 18, 24 ind m^{-2} (D12, D18, and D24 respectively). Crocodiles were fed a diet (68% protein and 4.63% lipid), at a ratio of 15% body weight (BW) per day adjusted daily based on growth and mortality.

Table 1. Survival, initial and final stocking density, decreased stocking density, final generated biomass, weight change, initial and final wet weight, initial and final length, length change, food consumption, food conversion factor (mean ± SD. of three replicates per treatment) of juveniles of *C. acutus* cultured in three stocking densities 12, 18, 24 ind m^{-2} (D12, D18, and D24 respectively).

Variables	D12	D18	D24
Survival (%)	63.33±5.77[a]	57.78±7.70[a]	65.00±10.00[a]
Initial stocking density (ind m^{-2})	12.00±0.00[c]	18.00±0.00[b]	24.00±0.00[a]
Final stocking density (ind m^{-2})	7.60±0.69[b]	10.40±1.39[b]	15.60±2.40[a]
Stocking density decrease (%)	36.67±5.77[a]	42.22±7.70[a]	35.00±10.00[a]
Final biomass generated (g m^{-2})	291.36±59.13[b]	421.11±87.72[ab]	578.52±156.21[a]
Initial weight (g)	102.50±0.87[a]	99.42±2.47[a]	103.67±3.94[a]
Final weight (g)	140.69±5.38[a]	139.93±6.46[a]	141.94±15.24[a]
Weight change (g ind^{-1} d^{-1})	0.89±0.13[a]	0.94±0.17[a]	0.89±0.35[a]
SGR	0.74±0.10[a]	0.79±0.13[a]	0.72±0.25[a]
Initial length (cm)	32.53±0.15[a]	32.49±0.10[a]	32.63±0.16[a]
Final length (cm)	37.06±0.38[a]	37.44±0.64[a]	35.73±2.13[a]
Length change (cm ind^{-1} d^{-1})	0.11±0.01[a]	0.12±0.02[a]	0.07±0.05[a]
Food consumption (g ind^{-1} d^{-1})	11.90±1.09[a]	10.87±0.55[a]	11.30±0.85[a]
Food conversion factor	13.50±1.05[a]	11.73±1.68[a]	13.85±4.41[a]

Different superscripts per variable show statistical differences among treatments ($p<0.05$).

Ganswindt (2012) reported that stress is a leading cause of death in captive grown *Crocodylus niloticus*, which was identified as a syndrome that threatens the physiological and physical integrity. The author attributes this problem to social conditions, such as stocking density, mating season, and environmental conditions such as temperature fluctuations and availability of food resources. Pérez *et al.* (2009) reported that excessive noise is a stressor causing mortality in early-stage crocodilians, while Brien *et al.* (2014) attributes the leading cause of mortality in captivity to a growth retardation syndrome (GTS), which can occur due to various factors, including various aspects of breeding, such as stocking density. The GTS leads to starvation and immune decline, conditions that eventually caused mortality in his study. In general, these factors can only lead to a stress response when conditions are poorly controlled. In the present study, temperature and food control were maintained to rule out these sources of stress. The mortality reported in the present project was possibly related to the inherent mortality rate in the early stages of crocodiles due to the initial handling weight of the crocodiles; the latter has been reported as a causal factor of death in early-stage *Crocodylus porosus* in captivity (Brien *et al.*, 2014). These values could explain the similarity in overall survival reported in the present study.

The absence of significant differences in final weight among the treatments in the present study is consistent with that reported by Brien *et al.* (2016). These authors tested four different densities (3.3, 6.7, 13.3, and 20 ind m^{-2}) in the early stage of *C. porosus* and reported that 6.7 ind m^{-2} grew faster than (6.12±1.24 cm) the density of 20 ind m^{-2} (2.33±0.63 cm). These results differ with Poletta *et al.* (2008), in an experiment testing three densities (8.3, 16.66, and 25 ind m^{-2}) for three months in the early stages of *C. latirostris*. These authors reported significant differences between higher and lower density, and obtained approximately 60 g and 5 cm total gain with a high density and approximately 150 g and 12 cm total gain in the low density. Pérez *et al.* (2009) reported the influence of two diets (marine fish and a mix of marine fish, beef liver, and beef lung) in the early stage of *C. acutus* in a period similar (50 d) to the present study; however, with different density (4 and 10 ind m^{-2}). The authors found significant differences in total length (17.2±0.8 cm) and wet weight (167.4±7.9 g) and the best performance in low density and a mix of marine fish, beef liver, and beef lung.

The results obtained in the present study showed that the stocking density did not influence the growth and survival of the crocodiles under the experimental conditions described. Several authors suggest that there may be an "optimal" density for the culture of the early stages of crocodilians. However, the optimum value is different for each species, which depends on the culture conditions and handling. Poletta *et al.* (2008) reported for *Alligator mississippiensis* the optimum stocking density at 10 ind m^{-2} during the first year of life and a range of 10-17 ind m^{-2} for *Crocodylus johnstoni* in the first three months of life. The National Resource Management Ministerial Council (2009) advises that in the first months of life, the stocking density does not exceed 10-15 ind m^{-2}.

Evidence of the optimal stocking density in crocodile culture is still under research. The results of the present study agreed with Meraz *et al.* (2008), who also did not report significant differences in the effect of stocking density in the early stages of *C. acutus*; however, they used lower densities. Other authors such as Pérez *et al.* (2009) and Hernández-Hurtado *et al.* (2012) reported better growth in the same species using lower densities (4.1 and 0.61 ind m^{-2}, respectively).

In the production of this species in early stages it is convenient to maintain a higher density per area unit to optimize its performance, although it is necessary to generate knowledge so that the conditions can be established in which a decrease in density can be carried out to continue with the culture. The crocodile farming industry requires strategies that reduce costs and labor and increase growth and profit margins, thus obtaining better profitability from infrastructure (Charruau *et al*., 2010; Blessing *et al*., 2014). Barrios-Quiroz and Casas-Andreu (2010) mentioned that culturing these animals is a costly process, and it is essential to know about the diet and adequate stocking density, which will allow maintenance a higher number of individuals per area unit to increase production. The generation of knowledge on culture techniques for *C. acutus*, such as the determination of the optimal stocking density for each developmental stage, could allow a better production in Mexico through the implementation of Wildlife Management Units (WMUs). Besides, the WMUs are centers for the conservation of endangered or threatened species and can be an alternative for the generation of economic resources in rural communities, within the framework of legality (Gallina and Escobedo-Morales, 2009; Retana-Guiascón *et al*., 2011; Álvarez-Peredo *et al*., 2018).

In some other crocodilian species, the economic and social conditions that exist in their range deplete the natural populations. Therefore, captive breeding is an essential tool to avoid overexploitation and support the species' repopulation. Barrios-Quiroz and Casas-Andreu (2010) mentioned that density influences individuals' growth in a considerable way. It is preferable to use newborn individuals since they present a better response than animals extracted from wild populations, in which there is higher mortality caused by stress.

The growth rate in wild crocodile populations is a vital parameter because if the animals grow faster, they are less predated, and survival increases (Charruau *et al*., 2010). In agreement with the previously mentioned study, growth is a determining factor in the fertility of individuals, since through this parameter age can be estimated, and therefore, appropriate decisions regarding the management, conservation or exploitation of wild populations can be made, which could also be applied to crocodiles in captivity (García-Grajales *et al*., 2012).

The diet we used with the combination of beef liver, fish, and dog food was similar to that used by Barrios-Quiroz and Casas-Andreu (2010) in the early stage of *Crocodylus moreletti*. These authors reported different diets, all constituted by mixtures of red meat, chicken liver, or fish. As a result, weight increased at the end of the experiment (9 months), approximately 78.42 g with the diet of red meat, chicken liver, and fish. Comparing our study with Barrios-Quiroz and Casas-Andreu (2010), in the present study a higher growth was obtained in 43 d (around 40 g) in the best treatment, compared with the 78 g obtained in 270 d in their study. Brien *et al*. (2016) mentioned that the growth rate in *C. porosus* decreased significantly after 21 d, and most of the offspring either lost or maintained their weight. Contrarily, Brien *et al*. (2014) indicated that newborns' growth rate can be extended 90 d or more, depending on diet quality. Therefore, it is possible to expect a similar effect in the early stage of *C. acutus*, since in the present experiment, the weight gain was constant.

Hernández-Hurtado *et al*. (2012) evaluated diets with different proportions of fish and beef liver in the early-stage of *C. acutus*. These authors indicate that the treatment with a higher percentage of beef liver obtained a growth of approximately 211 g in 5 months. Thus, the inclusion of beef liver was positive for the offspring, suggesting that the fat content allows the crocodiles to grow and survive as well as to withstand the effects of heat stress. On the contrary, Barrios-Quiroz and Casas-Andreu (2010) reported that diets with chicken and beef liver generate lower growth rates and consider that the inclusion percentage of chicken and beef liver can be a determining variable. Brien (2015) observed that the fish-based diet generates higher growth in length, and red meat increases weight. However, Pérez *et al.* (2009) reported that, although there is a high effectiveness in fish diets it is not recommended to supply said diet for a period longer than three months. In addition to the differences between one diet and another, the food proportion must be considered according to the weight of the individuals. The typical juvenile crocodilian will consume a proportion of food between 15 and 20% of its body weight per week, at a constant temperature of 32 °C, and once they reach the sub-adult stage, they only need to consume 8-10% per week (Blessing *et al.*, 2014). Different diets and factors that influence the growth of these animals have been reported. However, it is necessary to generate knowledge about the formulation of diets for the early stages of *C. acutus*.

CONCLUSIONS

Based on the results of the present study, it can be concluded that under the experimental conditions, there are no significant differences in stocking density in the interval from 12 to 24 ind m^{-2} and it is recommended to grow juveniles of *C. acutus* at a density of 24 ind m^{-2} to optimize the infrastructure's profitability. It is essential to conduct more research on the stocking density in juveniles depending on their weight and length, environmental and management factors, and the transition phase to decrease the stocking density according to their growth.

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