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Study of the Reproduction of *Cambarellus montezumae* (Saussure, 1857) Under Different Sex Relations

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Abstract

The crayfish *Cambarellus montezumae* (Saussure, 1857), is one of the endemic freshwater decapod crustacean species with the largest area of distribution in Mexico (Villalobos, 1955). This species is registered in the closed basins of the Neovolcanic Axis from Puebla to Jalisco, that is, along the Lerma-Santiago-Chapala system. For the present study, crayfishes of the species *Cambarellus montezumae* were collected in the Xochimilco Canals. We distributed 120 mature females and 48 F1 males (reproductive form) in 9 plastic tubs of 0.54 × 0.34 × 0.14 m with individualized PVC shelters, constant aeration and a temperature of 18°C. Three handling densities were tested: D1 (8 org/m^2), D3 (16 org/m^2), D6 (28 org/m^2), which will represent 4, 12, 24, females/m^2, and a sex ratio (Male : Female) of 1: 1, 1: 3 and 1: 6. It was appreciated that there was no fluctuation of temperature throughout the experiment. The statistical analysis in females did not indicate significant differences in the parameters of initial weight, final weight, final length. The differences were found in the parameters of weight gained in percentage, Specific Growth Rate, Food Conversion Rate, initial length, percentage of spawning and the number of eggs per female.

Keywords: *Cambarellus*; Reproduction; Spawning; Density

Introduction

Cambarellus montezumae (Saussure, 1857) is one of the endemic species of sweet water decapod crustacean with greatest area of distribution [1], this species is recorded in the closed basins of the Neovolcanic axis that extends from Puebla to Jalisco, that is, along the Lerma-Santiago-hapala system. Crayfish, in general, have successfully invaded a wide variety of habitats, and have been distributed on all continents, in bodies of fresh water, lotic, lentic or hypogeal. They live in both temperate and subtropical climates, which is why they are the most important, large and long-lived members of the freshwater macrobenthic communities [2,3].

The importance of crayfish is based on three fundamental aspects, the ecological, the nutritional and the economic. Economically, in the local markets, it represents a profit of 1 dollar per ration for consumption, food rich in protein, and 1/4 of dollar per unit to be used as live food in aquarium. In particular, due to the overexploitation of these crustaceans in the Valley of Mexico, a decrease in the natural populations of species such as *Cambarellus montezumae* has been observed [4,5]. Another element that has affected the vulnerability of the species is the overexploitation of water resources in the environments that host them. The main impacts that water bodies have had are the over extraction of volumes for drinking water consumption, the interception of fresh water intake and the discharge of wastewater. The combination of some of these impacts has resulted in the freshwater environment losing the necessary quality causing the reproduction rate of *Cambarellus montezumae* to be greatly reduced.

Within the aquaculture activity, having a brood-stock bank turns out to be very important because it provides the necessary breeding stock, which will be used for various purposes (sale, consumption, repopulation, etc.) [6]. In order to generate high efficiency reproductive organisms, the male-female relationship (M: F) that allows the greatest number of spawned females must be established, as well as determining the optimum density, which does not affect its growth and avoid a high mortality, since the effect of density on the reproduction of the crayfish is little known [7,8].

The objective of this research was to carry out studies in the process of controlled reproduction, determining the optimal M: F sex ratio for an efficient reproductive management in captivity and relating it to the quality of the water that houses the organisms, by following some of the physicochemical parameters (pH, temperature, O_2) and thus achieve the consolidation of a brood-stock bank that allows production alternatives to the habitants of central Mexico.

Materials and Methods

Crayfish of the species *Cambarellus montezumae* were collected in the Canales de Xochimilco with location 19° 17' 06" N; 99° 06' 07" O. Under an experimental design of randomized blocks with two repetitions, during 120 days, 120 mature females and 48 F1 males (reproductive form) were distributed in 9 plastic containers of 0.54 × 0.34 × 0.14 m with shelters individualized PVC, constant aeration and a temperature of 18°C. In this system three handling densities were tested: D1 (8 org/m^2), D3 (16 org/m^2), D6 (28 org/m^2), which represent 4, 12, 24, females/m^2, and a sex ratio of 1: 1, 1: 3 and 1: 6. (Male: Female) respectively. Water temperature and dissolved oxygen (DO) concentration were recorded daily with the help of a YSI Model 55 Oximeter (Yellow Spring Instrument, Ohio, USA), and the pH each week with a Lauka digital potentiometer, model 8010 (Orion Research Inc., Beverly, MA, USA).

The dead organisms and the spawned females were replaced to

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maintain the constant density, as well as the males that moved to non-reproductive form (FII) were replaced by FI reproductive males.

The growth was estimated based on the weight and the total length with biometrics that were made every fifteen days. The feed consisted of feed formulated for shrimp Camaronina 35 of Purina® (35% protein). In addition, the following parameters will be estimated according to [6]:

- Survival (%)=100 ((Initial number-Final number)/Total number)
- Weight Gain=(g) (Final weight-Initial weight)
- Weight Gain (%)=100 ((Final weight-Initial weight)/Initial weight)
- Length Increase (%)=100 ((Final length-Initial length)/Initial length)
- Specific Growth Rate (%/day)=100 (Final Weight loge-Initial Weight loge)/time)

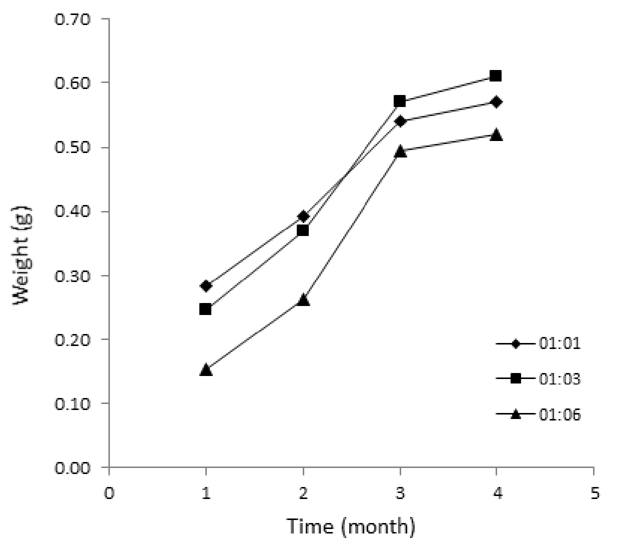

Figure 1: Growth in weight of females.

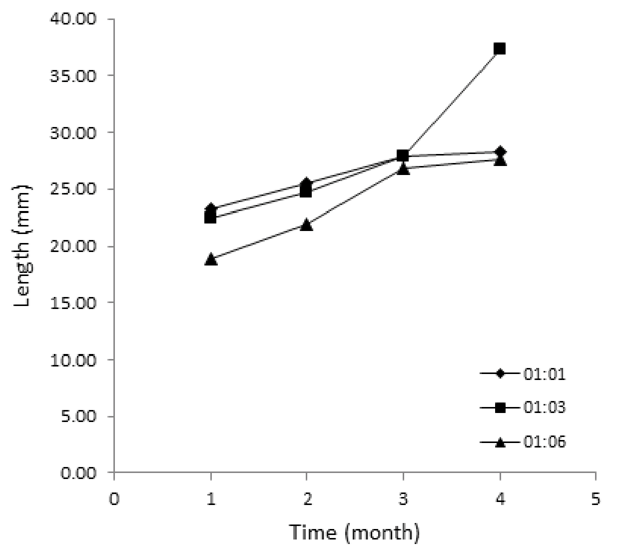

Figure 2: Growth in length of females.

- Food Consumption (g)=(Food supplied-food remaining)
- Food Conversion Rate=(Feed Intake/Weight Gain)

The results of survival, growth, maturity and spawning were compared using a one-way analysis of variance (ANOVA) with a level of significance of 95%. The differences between the means were established by other results of a multiple range test of Tuckey and data were normalized using the arcsine function when necessary [9]. Statistical Analysis Software, SAS/STAT V 4.1 was used for the statistical evaluation.

Results

In Table 1, the record of the average temperature and oxygen per experiment chamber and the general average of the treatment is

Treatment	Average temperature by containers (°C)	Average (°C)	Average (mg/l)
Sex ratio 1:1			
Container 1	22.56	22.86	4.8
Container 2	23.11		
Container 3	22.91		
Sex ratio 1:3			
Container 4	22.55	22.35	5
Container 5	22.25		
Container 6	22.24		
Sex ratio 1:6			
Container 7	22.51	22.76	4.6
Container 8	22.75		
Container 9	23.04		

Table 1: Temperature and oxygen record by experimental containers and by treatment throughout the experiment.

Parameters	Treatment			
	1:1	1:3	1:6	± SE
Female*				
Initial Weight (g)	0.28^{a}	0.25^{a}	0.20^{a}	0.042
Final Weight (g)	0.57^{a}	0.61^{a}	0.52^{a}	0.048
Survival (%)	66.66^{a}	75.33^{a}	76.66^{a}	8.144
Weight Gain (g)	0.29^{a}	0.37^{a}	0.37^{a}	0.031
Weight Gain (%)	108.99^{a}	165.99^{ab}	252.95^{b}	31.956
Specific Growth Rate (%/day)	0.81^{a}	1.05^{ab}	1.40^{b}	0.133
Food Conversion Rate	1.04^{a}	1.29^{ab}	1.74^{b}	0.125
Initial Length (mm)	23.25^{a}	22.48^{a}	20.84^{a}	0.995
Final Length (mm)	28.3^{a}	37.2^{a}	27.6^{a}	4.909
Length Gain (mm)	5.08^{a}	6.40^{a}	6.87^{a}	0.857
Length Gain (%)	21.98^{a}	28.59^{a}	36.14^{a}	4.231
Spawning (%)	8.33^{a}	8.33^{a}	12.5^{b}	0.0
Number of eggs by female	11.25^{a}	12.75^{b}	19.83^{c}	0.0
Male*				
Initial Weight (g)	0.34^{b}	0.23^{a}	0.17^{a}	0.024
Final Weight (g)	0.59^{a}	0.56^{a}	0.52^{a}	0.052
Survival (%)	100^{a}	75.0^{a}	91.66^{a}	9.622
Weight Gain (g)	0.25^{a}	0.34^{a}	0.35^{a}	0.058
Weight Gain (%)	73.1^{a}	151.8^{a}	226.1^{a}	45.711
Specific Growth Rate (%/day)	0.6^{a}	1.0^{a}	1.23^{a}	0.174
Food Conversion Rate	1.32^{a}	1.56^{a}	1.92^{a}	0.322
Initial Length (mm)	24.0^{b}	22.0^{ab}	20.7^{a}	0.474
Final Length (mm)	27.58^{a}	27.47^{a}	27.14^{a}	0.742
Length Gain (mm)	3.58^{a}	4.89^{a}	5.47^{a}	1.082
Length Gain (%)	15.04^{a}	24.81^{a}	23.80^{a}	5.250

Table 2: ANOVA of different parameters recorded in three different sex ratios.

appreciated; where it can be observed that there was no fluctuation of the temperature during the whole experiment and oxygen was at an average of 4.8 mg/l. The statistical analysis in females did not indicate significant differences in the parameters of initial weight, final weight, final length. The differences were found in the parameters of weight gained in percentage, specific rate of growth, feed conversion rate, initial length, percentage of spawning and the number of eggs per female.

In females, a rapid weight gain was observed in the D6 treatment (28 org/m^2), which, although it was the organisms that started the experiment with a short stature, was not so in the weight, which statistically did not present significant differences. Regarding the initial length of the organisms of D6, the organisms had a size of 18 mm being the smallest; however, they achieve equal final size to the organisms of the other treatments (Figures 1 and 2).

The results obtained for the feed conversion rate show values close to one which indicates that the crayfish are efficient organisms in the absorption of the food, the highest value was in the ratio 1: 6 where a greater waste of food was observed (Table 2).

The spawning percentage was higher in the third treatment, where the relation in which the highest proportion of females was found, was placed; although in treatment one and two spawning were observed from the second month, in the third spawning occurred until the last month.

For the number of eggs per female, differences were observed among all treatments, where the highest and lowest number of eggs per female occurred in treatments three and one respectively.

In males, the statistical differences were observed in the initial weight and length parameters. This is due to the fact that in the collection of organisms in the natural environment, no crayfish with homogenous sizes were extracted and the larger organisms were used in the first two sex relations.

Discussion

The density is undoubtedly a factor that affects the growth and survival of the crayfish and consequently the yield per production area. This inverse effect reported in several studies has indirectly influenced the use of low densities even for reproduction, in order to minimize the stress generated by social interaction, territorial restriction, cannibalism, limitation by food or by the indirect effect on others. parameters such as water quality [10].

In *Cambarellus montezumae* there is no record of its reproduction in controlled systems, so in this study two factors that are critical for its control are observed:

a) That the number of juvenile organisms is adequate to achieve the greatest number of mature females and with spawning.

b) That the sex ratio favors reproductive success.

In this respect [5,11] experimented with three densities in *Cambarellus montezumae* (77, 154 and 231 crayfish/m^2), observing that the best density to breed these organisms is that of 77 organisms/m^2 since they were the ones that reached the largest sizes. In this work it was found that the highest density condition (28 org/m^2) obtained the best indicators of growth in weight and height. However, it should be clarified that the density worked here is less than half, in the one studied in the works referred to (77 org/m^2).

The density also had a positive effect on spawning and the number of eggs per female, according to our results it is observed that the best relationship was found in D3 (1: 6). As the density increased, the spawning rate increased from 8% to 12%. In *C. quadricarinatus* the number of spawnings per area was also increased from 2 with 10 org/m^2 to 4 with 20 org/m^2 [12]. Despite the relationship found between density and spawning, no synchrony process was observed in the maturation or spawning of the females, which caused the females to mature at the same pace and spawn at the same time. Timing in maturation and spawning is important for controlled reproduction as it is very advantageous for production planning [13], but in this species as in other Australian species, maturation did not synchronize; neither did the spawning synchronized.

This asynchronous behavior under controlled conditions where the percentage of spawning does not exceed 50% of the population, can be related to a great variation between individuals, but can be improved up to 90% using females in the same intermolt stadium as proposed [13]. The density also seems to affect the maturation of the males. At the beginning of the experiment it was observed that all the males in the lowest density 1: 1 changed to non-reproductive form II within the following 24 hours after having placed them in the system. These were exchanged for form I males, which moved back to form II. It was then decided to use freshly molded form I males which were maintained until the end of the experiment without moving. However, it is necessary to specify this observation in future works.

The other aspect that determines the viability of captive breeding is feeding; among the authors who have done work on the type of diet of crayfish for their culture is Latournerié et al. [14], which tested the feeding of juveniles of *Cambarellus montezumae* with a ensilage of *Egeria densa* measuring the growth, survival, production and efficiencies of energy transfer, concluding that the use of enriched plant detritus presents conditions that are favorable for the production of brood hatchlings in extensive farming systems. Our results show that the feed conversion rate is close to one when the density is low, however, in growth this better yield is not appreciated, since there were no statistical differences in the increase in weight and length.

In another trial [15], evaluated the growth of juveniles *Procambarus* (*Austrocambarus*) *llamasi* under 6 different commercial diets for farm animals and aquaculture. The lowest growth results were recorded for rabbit, pig, trout and tilapia diets; however, they can be recommended under extensive and semi-intensive cultivation conditions since they have the advantage of having a low price in the market and no supply problems.

In relation to the correlation between reproductive efficiency and water characteristics, we have that [16] evaluated the effect of three different temperatures on the growth, survival and spawning of the cowbird *Cambarellus* (*montezumae*) *patzcuarensis*; the preliminary results show a greater growth in the temperature of 22°C. In the mortality rate, the highest percentage of mortality was found in low density with 33%. The highest gonadal maturity and best percentage of spawning, until this moment, is observed in the temperature of high density with 12.5%. Given this situation, and taking into account the importance of its conservation, it is that experiments aimed at controlled reproduction have been carried out throughout the country and under different environments in order to achieve the reinsertion of populations in their natural environments. Being *C. montezumae* a species of easy adaptation to laboratory management [2], it has been considered as feasible for its reinsertion into the sweet aquaculture environments of the Valley of Mexico.

Conclusion

Although under controlled conditions it is still necessary to specify the effect of density. The maturation process of the crayfish seems to be linked to the number of animals per area as shown by the results in *C. montezumae*. This gregarious behavior must work by promoting the maturation of females and males by increasing the likelihood of encounter and therefore of reproductive success. In natural populations, the density increases in the breeding season as a result of the decrease of flooded areas and the migratory processes of the organisms due to the drying of the land [17]. The increase in density has a promoter effect on the natural reproduction of several species of crayfish as part of their reproductive strategies, as observed in *C. montezumae* under the managed ratio of 1: 6 (M: F).

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