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GROWTH OF *ARTEMIA FRANCISCANA* UNDER LABORATORY CONDITIONS PROVIDING LIVE AND INERT FOOD

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Abstract: Two different diets were compared to estimate the growth in size and weight, as well as the survival of *Artemia franciscana* ; the first only composed with cema and the other a combination of cema and *Pinnularia* sp. An intensive culture of Artemia was carried out under controlled conditions in four aquariums (3 L capacity), with a stocking density of 10 organisms/ml, temperature of 25±1°C and salinity of 60 ups. The organisms in each aquarium were fed "*ad libitum*" in the morning and afternoon. During the first six days, all the aquariums were provided with cema and from the seventh day, two of the systems were provided with a mixed diet of microalgae (*Pinnularia* sp .) and cema. A random sample of 180 organisms was measured and weighed for thirty days. The brine shrimp treated with the mixed diet grew faster than the cema ones, being significantly larger (average length of 0.89 cm), the latter lived less time and through counts every third day, which had a tendency to decrease reaching values less than 1% at the end of the experiment, in both treatments. Despite this, the survival rate of organisms fed the mixed diet was twice as high as that of animals fed cema. Therefore, sustaining the early stages of *Artemia* with a diet rich in carbohydrates and subsequently supplementing it with *Pinnularia microalgae* sp , favors growth, but not its optimal development that allows high survival.

One of the aspects to which aquaculturists have paid attention is that related to the adequate nutrition of the cultivated organisms. No artificial or supplemental food can replace live natural food, especially when it is grown in the right conditions to keep it free of pathogens and rich in nutrients.

Artemia sp , is the microcrustacean most used in aquaculture as live food for fingerlings of many fish and larvae of crustaceans in culture and also for its easy handling (Persoone *et al.,* 1980; Vinatea , 1995; Godinez *et al.,* 2004; monroig *et al.,* 2006).

This crustacean is in demand due to the size it presents in its different stages of development (cysts, nauplii and adults). From a practical point of view, when the cysts are dry and dehydrated, they can be stored for years, since the embryos are in a state of diapause. However, the form of newborn *nauplius has acquired greater use due to its high nutritional value, in terms of lipids (Torrentera and* Tacón, 1989); However, the use of adult animals also provides numerous advantages, since they contain a greater amount of protein than nauplii (60% protein), which is beneficial for the reproductive conditioning of cultured organisms (Versichele *et al.,* 1991).

As aquaculture has progressed in the world, the supply of cysts and their alternative products, such as live biomass, frozen, lyophilized, flakes, pellets, etc., have not managed to cover the great demand that currently exists (Sorgeloos *et al.,* 1986; Vinatea , 1999; Seoka *et al.,* 2007), only the consumption of cysts is above 1 700 tons per year worldwide (Sorgeloos *et al.,* 1991). More than 90% of the commercialization of cysts of this crustacean comes from the Great Salt Lake (Utah, USA), although this activity is only extraction. In the last five years, the supply of this product has been reduced, due to the fact that there is a decrease in the population of *Artemia* . spp . in the Great Salt Lake and to the varied environmental conditions, in such a way that harvesting in this lake is currently suspended (Castro *et al* ., 2001).

A wide variety of live food (microalgae) and inert food (wheat flour, rice, soy, etc.) have been used successfully for the cultivation of *Artemia* spp . (Castro *et al* ., 2003; Stottrup and McEvoy , 2003). The development of artificial diets is another aspect that has been intensively addressed with a view to

reducing production costs and guaranteeing adequate nutrition for organisms. Among the indicators used by researchers and aquaculturists to evaluate larval production in experimental and productive systems, are survival, growth in size or weight, and the quality of the product determined by the response to stress tests. Among the latter, one of the most used because of the speed with which it is performed and does not require specialized equipment, is the salt stress resistance test (Castro *et al.*, 2993; Palacios and Racotta , 2007).

Artemia spp . It has a cosmopolitan distribution, it is typical of aquatic habitats with high salinity, which are widely distributed in the world. To date, its presence has been detected in 44 countries in 3000 localities of natural sites or as man-made salt pans (Sorgeloos *et al.,* 1986).

In Mexico there are 17 saline sites where this small crustacean can be found; 14 zones in thalassohaline coastal waters , which is distributed in 17 sites in salty coastal and inland water bodies under specific salinity and temperature conditions, so each of the populations can vary considerably with respect to physiological responses to make against the conditions that prevail in the different aquatic environments (Castro *et al.*, 2010). The remaining three sites are inland water habitats whose main component is not sodium chloride (athalosohaline). For example, Cuatro Ciénegas, Coahuila has water with sodium phosphate (Castro *et al.,* 2001).

Wind and waterfowl are considered the most important dispersal vectors; however, man has been responsible for inoculations in various places (Vinatea , 1995). The distribution and survival of *Artemia populations* sp , are strongly dependent on salinity (the concentration and ionic composition of their environment), as well as temperature.

It is a euryhaline organism found in Spanish salt mines with brine at 330 parts per thousand (Amat, 1985). The lower limit in the natural environment is, in most cases, determined by the presence of predators, which are abundant in salinities below 45 parts per thousand (UPS). This crustacean can tolerate temperatures from 6 to 35 °C, being related to the characteristics of each geographic strain. In relation to the pH of the *Artemia medium* , it is established in environments that oscillate between neutrality and relative alkalinity, the optimum being between 8 and 10 units.

Artemia franciscana (Kellogg 1906) *,* is an obligate non-selective filter feeder, its food is basically composed of microalgae that are present in hypersaline natural environments such as *Chateoceros , Dunaliella , Tetraselmis, Oscillatoria , Chlorella* , etc. Although microalgae are considered the most suitable food, they cannot be valued as the only food, especially when cultivated intensively; because in large-scale production of biomass, it is not economically profitable due to the large volumes required (Tizol , 1994). For this reason, the use of microparticulate foods such as yeasts, rice bran, corn, etc. has been selected (Vinatea , 1999).

The objective of this work was to analyze the individual growth expressed in total length of the population of *A. franciscana* providing two diets: live food (*Pinnularia* sp) and inert (cema), to determine differences in growth and survival, under controlled conditions of temperature and salinity.

MATERIAL AND METHODS

HATCHING PERCENTAGE

The percentage of hatching was determined for the two concentrations of salinity proposed in the Zaragoza Experimental Aquaculture Unit. This value was obtained by counting the number of

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nauplii, which emerge from one hundred cysts; it was convenient to avoid the presence of impurities: empty shells, sand, salt crystals, insects, etc. (Castro *et al* ., 2001). To do this, with the help of a microscope, 100 dehydrated intact cysts were chosen, placed in a Petri dish with saline solution at 25 psu, covered and left near a light source (20-watt focus) for 24 hours. At the end of the time, the nauplii were counted and separated. The procedure was the same for the concentration of 36 ups. It was recommended to repeat this operation 10 times so that the data is more precise. (Castro *et al* ., 2001). In addition, another test was also carried out in which the light exposure time was modified, for a photoperiod of 12 hours of light and 12 hours of darkness.

ECLOSION OF *ARTEMIA FRANCISCANA CYSTS*

Four batches of 0.5 g of cysts were hatched, after decapsulation treatment with a 1% sodium hypochlorite solution for no more than 10 minutes or until the coloration of the cyst changed from brown to orange. Once this happened, the cysts were immediately washed with water until the smell of chlorine disappeared, then a 0.02N sodium thiosulfate solution was added to eliminate chlorine residues (Castro *et al.,* 1991) .

Next, each of the batches was put to hatch in four tubular containers (1L), provided with an exit key; with water at a salinity of 36 ups. These were placed in a "bain-marie" to maintain the temperature at 25±1°C. By means of an air pump, oxygenation was constant and from the bottom of each container. A photoperiod of 12 hours of light and 12 hours of darkness was applied to these systems for 36 hours.

After 36 hours, the organisms that hatched from each of the tubular containers during this time were collected; For this, oxygenation had to be suspended and a focus was also installed above each system, so that in this way the nauplii migrated towards the light and concentrated in the upper part, the empty shells as well as the unhatched eggs were deposited at the bottom, in such a way that when opening the stopcock it was possible to eliminate the largest amount of sedimented impurities, and subsequently extract the *Artemia concentrate* .

The nauplii collected from each container were transferred to four experimental units (4L). In the case of the cultivation of *A. franciscana* , the literature recommends that it be cultivated in salinities between 60 ups and 80 ups, since greater growth and survival are obtained in this interval. In this work it was decided to work with a salinity of 60 psu; By means of a heater with a 50W thermostat, the temperature was kept at 25±1°C and oxygenation was constant using air diffusers.

The organisms were fed "*ad libitum*" with two different diets: Cema and Cema and *Pinnularia* sp (Mixed). Food was supplied in the morning and afternoon. The first five days the four cultures were maintained with an emulsion of cema (0.3 ml; in two liters of salt water at 90 g/L add 300 g of cema and liquefy for one minute). From the seventh day, the feeding changed, two of the cultures began to be supplied with the *Pinnularia microalgae* sp (500,000 cells/ml), while the other two continued to be given the cema emulsion.

DENSITY, MORTALITY AND SURVIVAL

To evaluate these population characteristics, the number of cysts present in 0.5g was previously counted. This count was done three times.

Once the cysts hatched, the density of each experimental unit was monitored every third day; To do this, the culture was gently homogenized with a glass stirrer, then an

aliquot of 1ml was taken and placed in a Petri dish to count the number of organisms. This procedure was performed three times to obtain a more precise value. The monitoring of this parameter allowed knowing the final mortality and survival presented by each experimental unit.

GROWTH AND WEIGHT

These parameters were quantified daily for each system for 30 days. For growth, from the first day of life of the organisms, a random sample of the culture was taken and from it a subsample of 30 organisms, each of them was placed on an excavated slide and measured by means of a microscope provided. of a micrometer eyepiece, the measurement was made with a magnification of 4X.

The weight of the organisms was estimated by weight difference using an analytical balance, and resorting to the positive phototropism that this type of crustacean presents, a lamp was placed on top of the systems, so that the Artemia migrated towards the light source. and there they will concentrate. Immediately, an aliquot of 5 ml of the Artemia concentrate was taken, this volume was passed through the sieve and immediately weighed on the analytical balance with a precision of 0.0001g. This operation was performed three times for each trial. The individual weight of the organisms was calculated based on the number of organisms present in a volume of 5 ml.

GROWTH MODELING

The length-weight relationship was applied, which is a potential type relationship according to the expression $P = a\ L^{b}$, where P is total weight, L = total length and a and b are constants. To carry out the linearization of the relationship, logarithms were applied to both sides of the equation and through linear regression analysis with the method of least squares (Pauly , 1984), the values of the slope (b) and of the intercept (log a).

The values of the slope obtained in each of the previous regressions were analyzed in order to verify their equality or inequality with three and thus be able to define the type of growth. If b= 3 there is isometric growth, while if b≠ 3 the growth is said to be allometric (Ricker , 1975). A Student "t" test was applied (Pauly , 1984), to determine if there was a difference in growth depending on the type of diet consumed by the organisms.

GROWTH RATES

The growth rate was evaluated for 30 days. The samplings were carried out daily, taking a sample of thirty organisms, for each experimental unit. The total length (Lt) was used, which included the anterosuperior margin of the head to the base of the caudal furca . To determine the growth rate, the following exponential model was applied Y = (ae^{bt}), where Y is the total length in centimeters, e is the natural base, a is the initial size, b is the growth rate and t is the time in days.

In the first instance, it was established to use the von Bertalanffy growth model to model the growth and estimate the asymptotic size of *A. franciscana* ; however, applying it overestimated the value; For this reason, the Gompertz model was investigated and it was considered feasible to estimate the growth of this species, since some authors have used it in the growth of crustaceans. Therefore, the growth model applied was that of Gompertz (Ricker , 1979), whose expression is $L_{(t)} = L_{\infty} e^{-Be^{-kt}}$, where $L_{(t)}$= length at time "t", L∞= maximum observed length, B=maximum growth rate, k= intrinsic growth rate, where $L_{(t)}$ = length at time "t", L ∞= asymptotic length, b= instantaneous growth coefficient. For the linearization of the Gompertz model, the following should be considered $L_{\infty} = L_{max} - L_{min}$ either $L\infty = \frac{Lmax}{0.95}$, where Lmax and Lmin correspond to the maximum

and minimum lengths observed in organisms. Next, the algebraic clearance is carried out to establish the linear equation and obtain the constants a = ln B and b = k.

RESULTS

Two cultures of *Artemia franciscana were grown* , each with a replica, in aquariums (4L capacity) using artificial aeration and heaters, to keep the temperature controlled at 25°± 1°C. The cultures began with the hatching of Artemia franciscana cysts, provided by Dr. Jorge Castro Mejía, in charge of the Man and his environment laboratory, of the National Autonomous Metropolitan University, Xochimilco Unit.

Stocking density was 10 organisms/ml for each aquarium. From the third day after hatching, the organisms were fed " *ad libitum* " with an emulsion of cema, (by-product of bean grinding formed by bran, aleurone and part of the endosperm). From the seventh day, two of the aquariums changed their food for *Pinnularia* sp (density 500 cells/ml) and the other two systems were continued to be given cema until the end of the experiment.

PHYSICAL AND CHEMICAL PARAMETERS

The most important parameters in the controlled cultivation of Artemia are temperature, salinity and pH (Table 1).

The temperature observed in the cultures fed with the mixed diet was in a range from 25 to 27°C, and for the cultures maintained with cema it varied from temporary between 25 to 26°C; When applying the Kruskal-Wallis test, no statistically significant differences were observed ($H = 3.156$; $P<0.05$). The salinity initially established was 60 psu. In the cultures with mixed diet, a minimum of 63 ups and a maximum of 67 ups were recorded, for the systems maintained with cema, the minimum value was 62 ups and the maximum was 65 ups. However, salinity showed statistically significant differences in both treatments ($H = 13.960$; $P<0.05$).

The pH was maintained at a minimum of 9.4 and a maximum of 9.7 for systems fed the mixed diet. For the systems with cema, the minimum value of 8.9 and the maximum of 9.6 In pH, there were also statistically significant differences in time ($H = 20.633$; $P < 0.05$).

A Mann-Whitney U test was carried out to show if there are differences between the treatments and their replica. For salinity, statistical differences were recorded between both ($W = 114.500$; $P = 0.015$). Salinity in the systems fed with cema showed significant differences ($W = 10.500$; $P = 0.004$) and pH did not show statistical differences ($W = 60$; $P = 0.733$).

Crop/parameter	Minimum	Maximum	Average
Cema/Temperature (°C)	25	26	25.2
Cema/Salinity (ups)	62	65	63
Cema/pH	8.9	9.6	9.3
Mixed/Temperature (°C)	25	27	25.4
Mixed/Salinity (ups)	63	67	63.3
Mixed/pH	9.4	9.7	9.6

Table 1. Values of the environmental variables in each crop with different feed.

HATCHING PERCENTAGE

At this stage, the hatching percentage was calculated, proposing two salinities 25 ups and 36 ups. For this, 100 dehydrated eggs were counted, which were hatched in a Petri dish with a 25 psu solution and another 100 eggs in a salinity of 36 psu. The capsules remained closed and near a light source for 24 hours. Three replicates were made for each salinity. At the end of the 24 hours, the nauplii that had emerged from each box were counted.

The results of this trial revealed that when hatching the cysts in a salinity of 25 psu, hatching is minimal since only 15.6% was recorded, compared to the salinity of 36 psu in which hatching was obtained above the 80.6%. Taking these values into account, the highest salinity (36%) was used for the next experiments.

If the photoperiod is used with the beginning of 12 light – 12 darkness, hatching of 81% is produced; however, subjecting the cysts to a continuous light photoperiod causes some organisms to die; on the contrary, if it is applied and started with the photoperiod 12 dark - 12 light, apparently it does not affect them, however a lower hatching percentage of 72.4% is obtained.

SURVIVAL

Cema-fed crops. The survival values in this type of culture decreased as the culture was carried out. In figure 1, it can be seen that from the fourth day of the culture, 50% of the organisms had already died, by the seventh day the density dropped to less than 10,000 (1%) organisms, this behavior remained constant, until present a sudden mortality greater than 90% on day 13. The maximum survival value was 60,000 (53.51%) organisms and the minimum 280 (0.22%) organisms.

Crops fed with cema and *Pinnularia* sp (Mixed). In Figure 2, it can be seen that mortality was continuous throughout the study. On day 3, survival was 62,300 (51%) organisms and up to day 8 it dropped to 12,600 (20%). However, from day 9, the changes in the values of this parameter were not as radical as in the first days. The maximum value for the survival of this culture was 62,300 (51.68%) brine shrimp and the minimum was 420 (0.674%) organisms. Similar values were obtained for the replicates of each treatment.

INCREASE

Cema-fed crops. In figure 3, it can be seen that the average length of the organisms fed with cema on day 1, had a minimum value of 0.04 cm and a maximum of 0.05 cm, both cultures presented a similar behavior, until the third day where already there is a difference in the growth of the organisms. This point where the behavior begins to vary is just when the nauplii began to be fed (day 3).

The brine shrimp from the culture fed with cema were always larger. Statistical analysis (Mann Whitney U) revealed this pattern ($U = 69131$; $P = 0.05$). Regarding the duration of the cultures, one remained 11 days (cema) while its replica (cema R) continued for three more days (14 days), both lasting approximately half the time compared to the cultures fed with the mixed diet.

The maximum average length reached by the organisms of both aquariums until day 11 was 0.19 cm, the maximum size reached for the replica (cema R) until the end (day 14) was 0.23 cm.

Crops fed with cema and *Pinnulari* a sp (mixed). The growth of *A. franciscana* fed the mixed diet can be seen in figure 4. Although both systems were given the same type of food and proportion, there were variations. The brine shrimp in aquarium 2 (mixed) were always slightly larger than those in the replica (mixed R); the initial average length of these organisms had a value of 0.04 cm and 0.05 cm, respectively; from day 1 to 7 they presented a

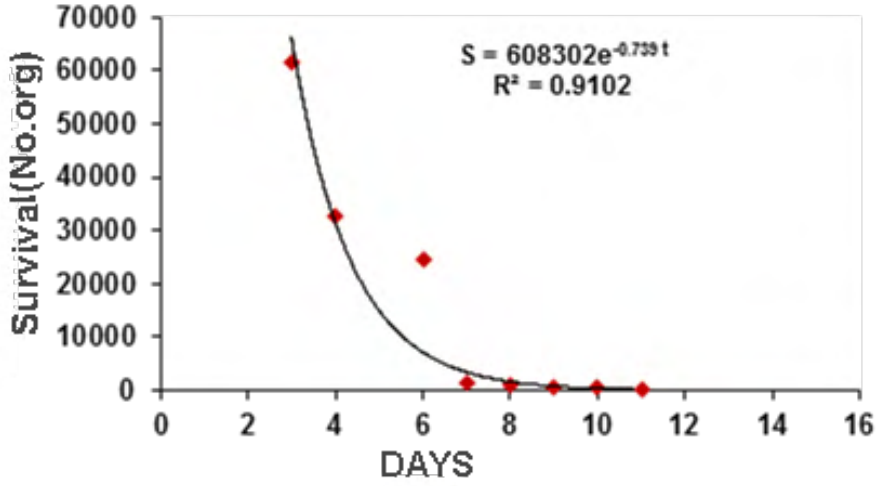

Figure 1. Temporal variation of survival in the culture of A. *franciscana* , fed with cema.

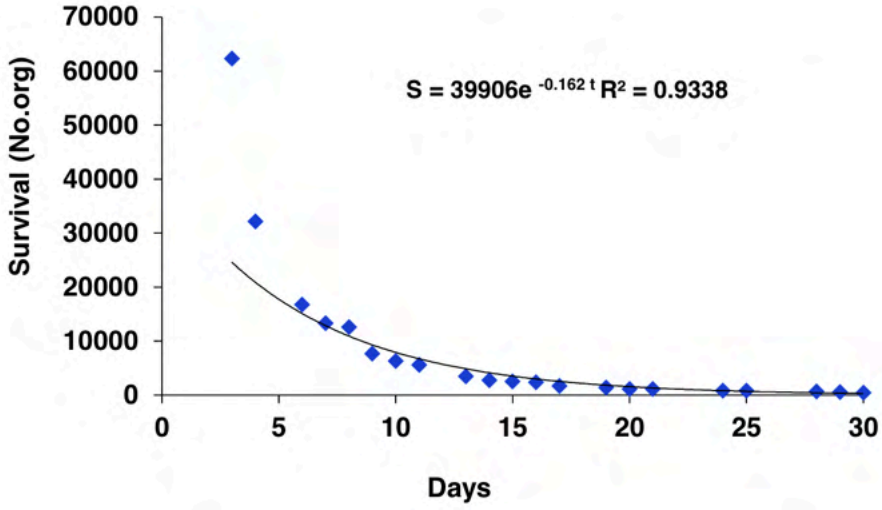

Figure 2. Behavior of survival in the culture of A. *franciscana,* fed with cema and *Pinnularia* sp.

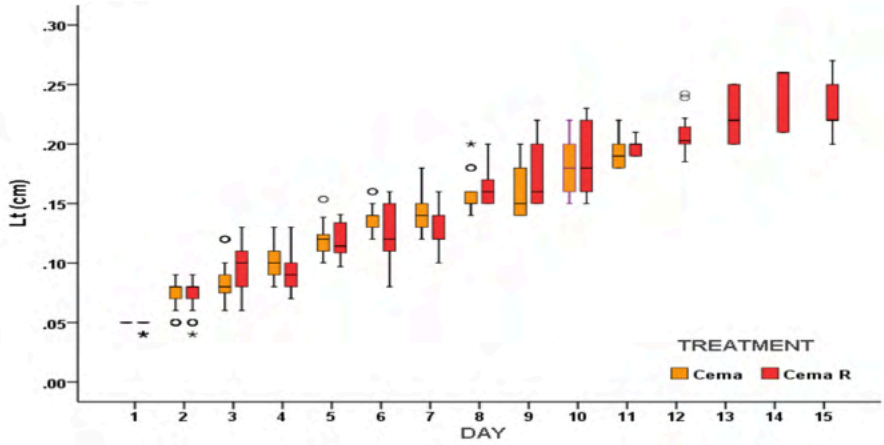

Figure 3. Variation of growth in length for the two cultures of *A. franciscana* fed with cema.

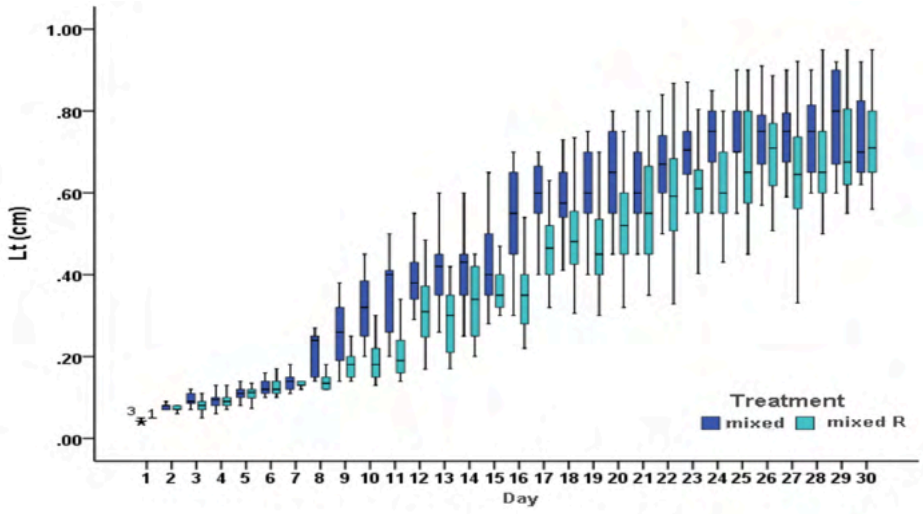

Figure 4. Variation in size growth for both cultures of *A. franciscana* fed with cema and *Pinnularia* sp . (Mixed).

more or less similar behavior, but from day 8, the organisms of aquarium 2 (mixed) had a more accelerated growth.

The maximum average length reached by the organisms of both aquariums up to day 30 was 0.77 cm for Mixta and the maximum reached for Mixta R was 0.71 cm.

GOMPERTZ GROWTH MODEL

In order to generate a model that better represents the growth of *Artemia franciscana*, under the conditions postulated in this study, the von Bertalanffy model was chosen; however, when it was applied, it did not fit the data obtained, since the average length of the brine shrimp was greatly overestimated. For this reason, it was decided to apply the Gompertz model ; Weymouth *et al* ., (1931; quoted in Gómez, 1994) mentions that this has been widely used for invertebrate organisms, frequently for crustaceans. When applying the Gompertz model, it was observed that it did adjust better to the data obtained and, therefore, better represented the growth of A. *franciscana.*

Cema-fed crops . The brine shrimp fed only with cema presented an initial average size, equal to that of the organisms that were provided with the mixed diet of 0.05 cm, the maximum size reached was 0.27 cm for 15 days of culture. Based on the Gompertz model, the estimated maximum length was 0.23 cm (Figure 5).

Crops fed with cema and *Pinnularia* sp (Mixed). The initial average size of the brine shrimp fed the mixed diet was 0.04 cm, the maximum size reached was 0.92 cm. Based on the Gompertz model, the estimated maximum length was 0.90 cm for 30 days of cultivation (Figure 6).

WEIGHT-LENGTH RATIO

The length-weight relationship for brine shrimp showed potential growth, obtaining different degrees of correlation. The value of the slope for the culture with cema was b=5.5994 (Figure 7) and that of the mixed culture (cema with *Pinnularia* sp .) with a slope value of b= 4.6739 (Figure 8), which indicates that the growth of organisms is different from isometry, that is, they have a non-proportional growth between the parts of the body, which means a allometric growth .

As can be seen in both figures, the greatest growth in size and weight was obtained by organisms fed on the mixed diet.

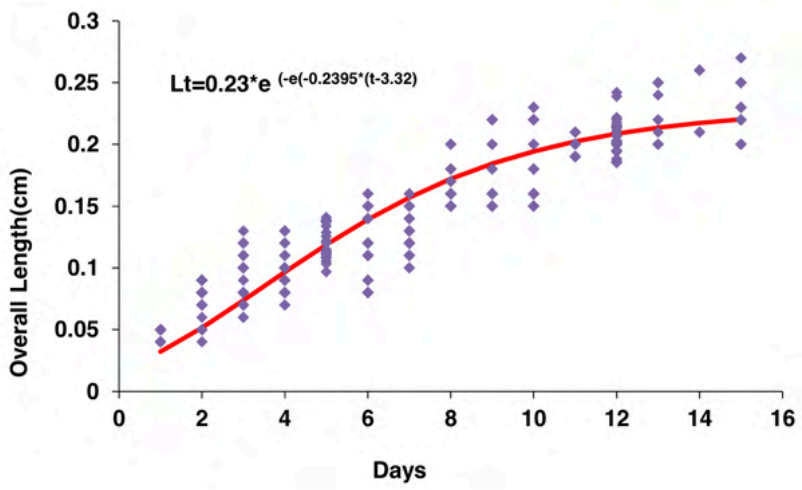

Figure 5. Gompertz model , to represent the growth of *A. franciscana* fed cema.

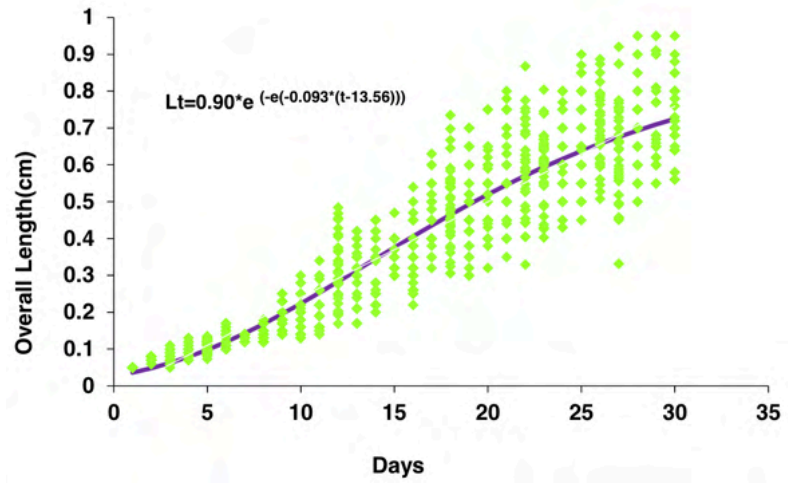

Figure 6. Gompertz model, to represent the growth of A. *franciscana* fed with cema and *Pinnularia* sp.

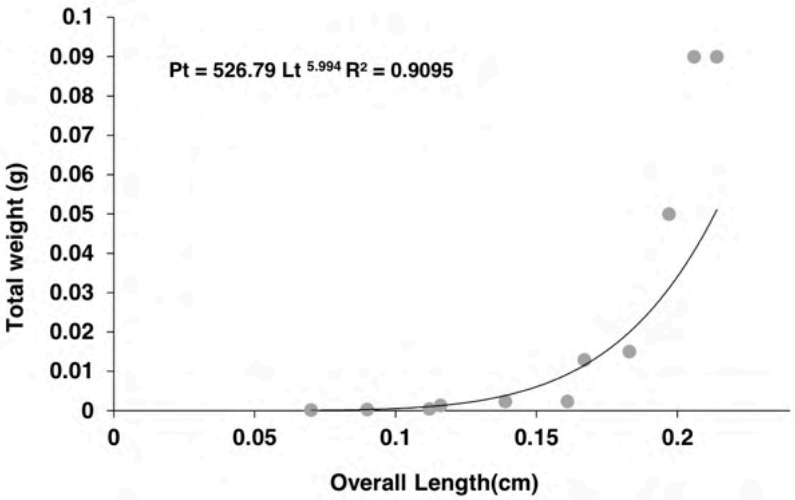

Figure 7. Length-weight relationship for the culture of *A. franciscana* fed with cema.

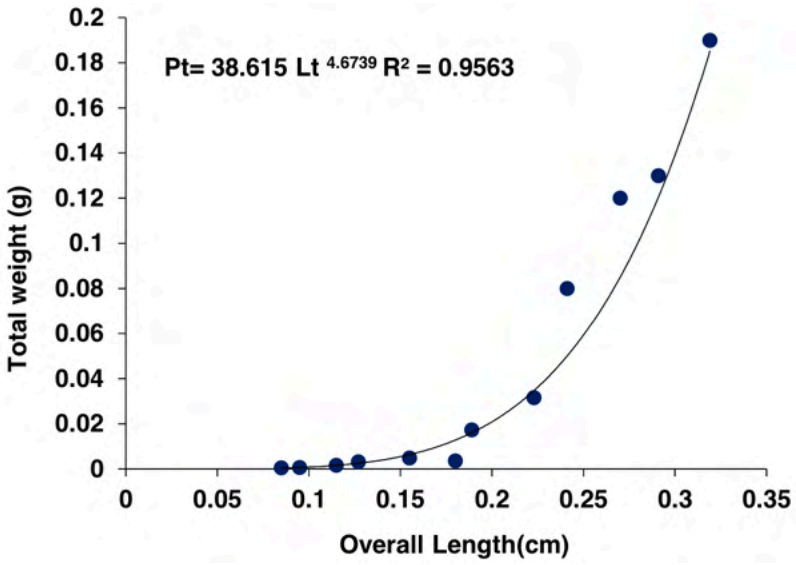

Figure 8. length ratio - weight for cultivation of *A. franciscana* fed with cema and *Pinnularia sp.*

DISCUSSION

The results obtained in this work are within the time indicated in the literature, which extends between 10 to 30 days as indicated by Sorgeloos *et al.* (1986), Wear and Haslett (1986), Correa-Sandoval *et al.* (1994).

The growth of aquatic organisms depends largely on the quality of the water, so to achieve good production, it is necessary to maintain the physical and chemical conditions of the water within the tolerance limits for the species to be cultivated (Arredondo and Ponce, 1998). In the specific case of *Artemia* , it is relevant to maintain temperature, dissolved oxygen, pH and salinity within certain intervals (De los Ríos, 2001).

Water temperature is one of the main factors that affect the physiological processes of organisms, including growth and reproduction (King, 2007). Dont and Lavens (1996) mention that for an adequate production of *Artemia franciscana* under controlled conditions, the temperature must be between 19°C and 25°C; Sarabia (2002), widens the range of this parameter and posits an optimum between 25°C and 27°C. In this experiment that was carried out, the temperature of the water had an average of 25.2°C to 25.4°C. Therefore, it can be considered that the experiment was carried out under adequate growth conditions.

The concentration of dissolved oxygen (DO) must be adequate for the survival and growth of organisms; the minimum concentration of this gas will depend on the species and the exposure time (Arredondo, 1986; Arredondo and Ponce, 1998). The critical concentration of dissolved oxygen for the case of *A. franciscana* is less than 2 mg/l, therefore it is necessary that the amount of oxygen is greater than this concentration (Dont and Lavens, 1996; Castro *et al* ., 2001).

Gilchrist (1956) mentions that when *Artemia is cultured* under marine conditions, in waters with salinities of 150% or more, where dissolved oxygen is often very low, hemoglobin allows *Artemia to* obtain enough oxygen to survive. , therefore, in the experiment that was carried out, by having constant aeration, it is very likely that it did not affect the growth of the organisms.

The pH of water is the result of the interactions of biotic and abiotic processes and is a measure of the acidity or alkalinity

of the water (Romero, 1999). Most organisms tolerate changes in pH within the range of 6.5 to 9, which is adequate for aquatic life to develop (Arredondo and Ponce, 1998; Gómez-Márquez *et al* ., 2014). For the cultivation of this crustacean, Castro *et al* . (2001), mentions that the pH must be kept between 8 and 10, while Dont and Lavens (1996) suggest that it be between 6.5 and 8.0 units. In the experiment, the pH value was considered slightly alkaline, due in part to the characteristics of the drinking water with which the study was carried out and, on the other hand, to the salinity used in the culture.

The salinity factor is one of the different physical-chemical variables that are of greater importance has on modifying the environment of organisms, due to its effect on the cycle of life and development, especially in those who live in aquatic environments; and much more in those who inhabit environments that present wide fluctuations, such as those who live in coastal lagoons and bodies of water where salt is produced (Castro *et al* ., 2010). Salinity is considered to be the main factor for a population of *Artemia* to be present or not in a given habitat. In a natural habitat, brine shrimp have been found surviving above 340 psu (Post and Youssef, 1977), although in reality the organism only survives, since all its physiological and metabolic functions are seriously affected. Dont and Lavens (1996) have suggested that salinity should be kept between 32 and 65 psu; Castro *et al* . (2001), recommends that it be kept between 60 and 80 psu, since in this way a greater survival of the organisms is obtained. Likewise, in order to achieve the highest hatching percentage, Castro *et al.* (2001), suggest that the production of this crustacean be carried out in the laboratory, at 36 psu of salinity, although it has been observed that the higher the salinity, the number of cysts also increases (Castro-Mejía *et al* ., 2014).

In the second stage of the work, once the nauplii were obtained, they continued their development by growing them at a temperature of 25°C and 60 ups of salinity, parameters similar to those used by Medina *et al* . (2007) where the reproductive response of two species *Artemia franciscana* and *A. persimilis was compared* .

One of the most complete works regarding the physiological response of different artemia species is that of Browne and Wanigasekera (2000), who used the species *A. franciscana, A. salina, A. sinica* and *A. persimilis* , cultivated at 15°, 24° and 30° C and combined with salinities of 60, 120, and 180 ups. The species of *Artemia* parthenogenetic, *Artemia sinica* and *Artemia franciscana*, obtained better growth and better production of nauplii and cysts when cultivated at 24°C and 120 psu of salinity; while *A. salina and A. persimilis* , obtain their optimum growth and reproductive characteristics at 24°C and a salinity of 180 psu. Hammer and Hurlbert (1992), observed that the juvenile organisms of *A. franciscana* grow very slowly and that the adults die in salinities below 38 psu, although Castro-Mejía *et al* ., (2011), mention that survival is inversely proportional to salinity

Regarding survival, Dhont and Lavens (1996) report values of 72% in cultures of *A. franciscana* with a density of 10 organisms/ml, fed with live microalgae. Similar values were obtained by Teresita *et al* . (2005), providing a mixed diet from day 2 to 6 with rice bran and from day 7 to 15 with the microalgae *Tetraselmis Suecica* .

Lora and Voltolina (2003), carried out an experiment in which the growth and survival of *A. franciscana* fed with three different portions of *Chaetoceros* sp and *Chlorella* sp. for seven days; at the end of the experiment, the average survival was in an interval between 89% and 100%, without any statistically significant difference. However, in the culture

of the organisms fed with the rations 23.4 and 46.8 mgl/l of *Chaetoceros* sp., these were significantly larger and heavier, compared to the organisms that were fed with *Chlorella* sp, with whatever the ration was.

Lora (2004) compared the growth through the energy balance of *A. franciscana* using the microalgae *Chlorella capsulata, Chaetoceros muelleri, Isochrysis* sp and *Nannochloropisis oculata* and obtained a survival between 85 and 90%, with all diets except *N. oculata* (31 to 45%). The growth in weight of the organisms showed the following behavior: *C. Muelleri* > *Isochrysis sp* > *C. capsulata* > *N. oculata* .

Contrary to what was previously expected, Castro *et al* . (2006), obtain a survival between 1.9% and 7.2% of *A. franciscana* , from the Juchitán Oaxaca salt mine, cultivated at a salinity of 60 g/l, fed with rice bran, in its early stages of development and from the fifth day they were given *Isochrysis galvana* and *Tetraselmis suecica* . Survival in this study was very low, even less than 1%, for both diets used. In all the reports cited above with the exception of Castro *et al* . (2006), all values are reported above this study.

With regard to food, it plays an important role in the growth and survival of organisms in culture. In this study, two diets were used, one based on cema and another mixed with cema and *Pinnularia* sp. At the beginning of the experiment, the four experimental units were given cema and from the seventh day on, two of them had their food changed for microalgae; coinciding with Castro *et al* . (2001), who mention that it is advisable to feed microalgae to brine shrimp from the seventh day.

Since this species requires abundant carbohydrates during the first days of life (Johnson 1980), probably the cema only satisfies the nutritional needs of *Artemia* during the first stages, but as they progress in their development, the nutritional requirements change and maintain them only with a carbohydrate-rich diet is insufficient to continue with its optimal development, which at the crop level is reflected in their survival.

Therefore, cema as the only food cannot be considered a suitable diet for *A. franciscana*, as demonstrated in this study, because the amount of energy obtained is not sufficient to sustain its optimal growth.

Regarding the mixed diet, Castro *et al* . (2001) mention that by providing a combination of green and brown microalgae, a better result is obtained, since the green microalgae supply proteins and pigments and the brown ones mainly provide carbohydrates and fatty acids.

Arriaga and Re Araujo (1997) and Lora and Voltolina (2003) using diatoms as food for this species, mainly with *Chaetoceros* sp., report good growth and survival; In this study, the brine shrimp fed with cema and microalgae presented larger sizes and better survival. However, no reference was found with which the results of this work could be compared.

Ribeiro and Da (1989), handled two types of food, the inert based on wheat bran and the other with *Tetraselmis suecica* and *Dunaliella salina* and conclude that for the cultivation of *A. franciscana* , the best results are obtained with *T. suecica*.

Therefore, the different survival values recorded can be attributed to the type of diet and the inequality in the nutritional requirements of the nauplii of different ages, this is due to the fact that not all the organisms hatch at the same time and also as they develop, their nutritional needs are changing, which in turn are related to changes in the ontogeny of the digestive system.

Another important factor that influences feeding is the maturation process of the organisms, Castro *et al* . (2001) report pair formation at 25 and 28 days feeding

this branchiopod with rice bran and fresh *Spirulina* , respectively. Arriaga and Re Araujo (1997), report that mating occurred between days 12 and 13. In this study, copulation was observed from day 25, but only in the cultures that were given the mixed diet. Therefore, it can be established that the type of food was decisive for these conditions to occur.

The crustacean *Artemia* has become very important today due to its high demand in aquaculture (Sorgeloos *et al* . 1986), because it represents one of the most widely used live diets in the farming of marine fish and crustaceans (De los Ríos, 2001).

In the present study, the population of *A. franciscana* fed with cema and the microalgae, reached sizes of 0.92 to 0.95 mm after 30 days of culture; however, when comparing the results obtained by De los Ríos (2001), who worked with two populations of *A. franciscana*, he obtained maximum growth values after 12 days of culture of 0.71 and 0.67 mm; These values are lower than those reported for the same time in this crop.

A possible explanation for this difference in growth could be that the environmental factors that influence the development of *Artemia populations,* especially in the individual growth of the organisms, is the predominant type of ion in the habitat, thus it is populations adapted to brines rich in chlorides, sulfates and carbonates, developing to the maximum in predominance of one of these three ions (Cole and Brown, 1967).

On the other hand, to represent the growth of *A. franciscana* by means of some growth model, in this study it was observed that the Gompertz model better fits the representation of growth in size for the species, since the von Bertalanffy and the logistic models register values below the maximum recorded during cultivation, therefore underestimated growth.

Ricker (1979) mentions that this curve expresses the decreasing rate of growth through the adult life stages of the fish and can be considered as the expression of the activity of two different and opposite types of regulatory factors during growth. In addition, Moreau (1987) cites that the Gompertz function is suitable for short-lived tropical species with significant changes in feeding from juvenile to adult stages.

Wear and Haslett (1986) cultured *A. franciscana* and observed that the model that best adjusted growth was the logistic one. They chose this model because the data reflected nonlinear behavior and also showed evidence of being better fit by this model, which includes an exponential phase, a declining gradient, and an asymptotic phase. The females grew to a mean length of 12 mm at temperatures of 17 and 26°C and salinities of 140 and 200 ‰ and the males measured 11 mm, higher values than those recorded in the present study.

Consequently, they mention that the ability of *A. franciscana to* grow rapidly and survive better in high salinities, as also mentioned by Castro-Mejía *et al* ., (2011), depends on the genetic adaptation in the biotope in which it is found. , since it has been observed that *A. franciscana* grows faster at temperatures from 20 to 28°C in salinities from 100 to 170 psu; furthermore, more than 90% of nauplii survive to maturity within this range and individuals can live for 5 months (Wear and Haslett, 1986).

Therefore, it is considered that the growth results obtained in salinities and temperatures are adequate, despite the fact that the recommendations suggest higher values than those used here. In addition, *Pinnularia* sp, may be another food option for this branchiopod, so more studies are required regarding the nutritional contribution of this diatom for the cultivation of *A. franciscana* .

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