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Effect of different salinities on the survival and reproductive characteristics of populations of *Artemia franciscana* Kellogg, 1906 from coastal and Inland waters of Mexico

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Abstract

The effect of salinity on survival and reproductive characteristics of four *A. franciscana* Mexican populations: Juchitán, Yavaros, San Luis Potosí, and Texcoco was evaluated. They were cultivated in 200 L beakers at 80, 100, 120 and 140 gL⁻¹ of salinity concentration, at 25 ± 2 °C, with continuous light and aeration and pH of 8-10. The organisms were fed *ad libitum* with rice bran (50 mL) and 1 L of *Tetraselmis suecica* and *Pinnularia viridis* microalgae. When organisms reach sexual maturity, were separated in 25 glass beakers (200 mL), one female and two males to determine reproductive characteristics. The results show that survival and reproductive characteristics values increase with salinity in all populations. This information is valuable to understand the adaptation patterns presented by each *Artemia* population and can determined the biological basis for exploitation in semi-intensive crops to assure the demand that this organism has in the aquaculture and aquarium industry.

Keywords: aquaculture, *A. franciscana*, reproductive characteristics, aquarium, salinity

Introduction

The crustacean *Artemia* inhabits aquatic environments of high salinity, which are widely distributed throughout the world and its composition can be sodium chloride or other components. They also can be found in evaporation or concentration ponds and crystallization pools, built by humans called salt factories [1, 2].

It has been seen that this organism is able to survive in water bodies with salt concentrations that oscillate above 340 gL⁻¹ and under laboratory conditions it can tolerate fresh water for several hours. On the other hand, the range of tolerance of salinity largely depends on the distribution of each populations of *Artemia* (bisexual and parthenogenetic) [3].

In recent years, this crustacean has played a central role in the development of aquaculture and mariculture as it is in demand due to the size it presents in its different stages of development (cysts, nauplii and adults), and are necessary for the growth and survival of many freshwater and marine species of commercial importance [4].

The distribution of populations around the world is in 505 localities [2]. Of these, 194 correspond to the American continent. Particularly in Mexico, its distribution is found in five sites in the Baja California peninsula; eight sites in the Pacific coastal area; two sites in the Mexico Gulf area; five sites in the Yucatan Peninsula and five sites in inland waters of the Mexican Republic [5]. This great plasticity to support different environmental conditions and physicochemical characteristics of its habitat, has allowed each population to present a variety of strategies and responses in their life cycle, morphology, reproductive and biochemical characteristics, which give particularities to each one of them [6].

Researches made with populations of *A. franciscana* in Mexico are about biometric and reproductive characteristics [7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17]. It should be noted that the works in the production of *Artemia* were made on the saline interval of 40-60 gL⁻¹ of salinity.

Therefore, the objective of this research was to evaluate the effect of salinity on survival and reproductive characteristics (pre-reproductive, reproductive, post-reproductive period, number of broods, interval between broods, nauplii and cysts produced by female per broods) of four Mexican populations of *A. franciscana* located in Pacific coastal waters and inland waters, which serves as the basis for an adequate management of natural populations as well as the development of sustainable, optimal crops of biomass production, cysts and even Desired

stages of development, such as those of nauplii and adults, managing to meet national demand in the aquaculture and mariculture industry.

Materials and Methods

Cysts of Mexican *A. franciscana*

This study was made at Live Food Production Laboratory from El Hombre y su Ambiente Department from Universidad Autónoma Metropolitana Xochimilco. Cysts from four

Mexican populations of *A. franciscana* were used, which were taken from the laboratory cysts bank (stored in a refrigerator at -10°C to maintain the process of dehydration and diapause in them).

Geographical location of the studied populations

The localities, abbreviation and geographic location of *Artemia* populations used in this research are presented in Fig. 1 and Table 1.

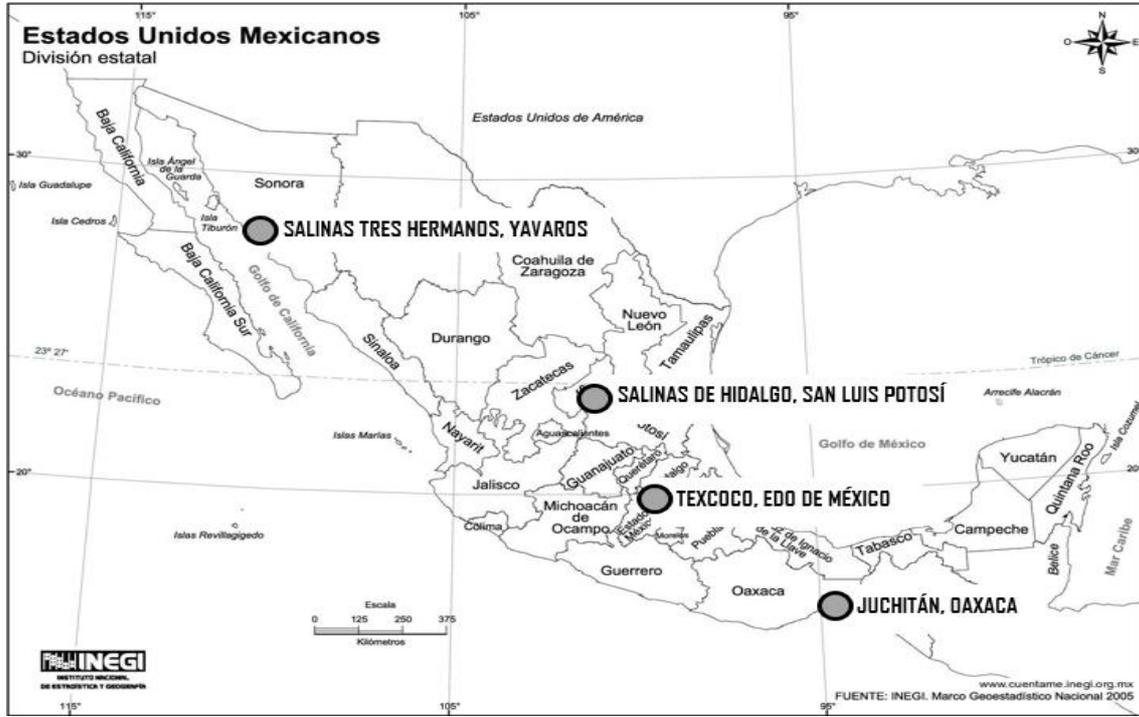


Fig 1: Geographical localization of Mexican *A. franciscana* populations at coastal and inland waters

Table 1: Mexican *A. franciscana* populations used in this experiment.

Locality zone	Abbreviation	Geographical coordinates
Pacific coastal waters		
Juchitán, Oaxaca	JUCH	16° 26' N; 95° 01' W
Salinas Tres Hermanos, Yaveros	YAV	26°41' N; 109°31' W
Inland waters		
Salinas de Hidalgo, San Luis Potosí	SLP	22°38' N; 101°43' W
Texcoco, Estado de Mexico	TEX	19° 32' N; 99° 00' W

Feeding organisms

The organisms were fed every third day with 50 mL of rice bran (300 g 4 L⁻¹ of water at 100 gL⁻¹ of salinity) and one liter of each microalgae: *Tetraselmis suecica* (Kyllin: Butcher) and *Pinnularia viridis* (Cleve) at a concentration of 500 x 10³ cells mL⁻¹ [18].

Experimental design

One gram of cysts from each population, were hatched in 4 L containers with 40 gL⁻¹ of salinity, with a pH of 8-10; temperature of 25 ± 2 °C and permanent aeration and illumination [18]. The nauplii hatched from each population were harvested and transferred to four containers of 200 L capacity previously washed and disinfected with 10 mL of sodium thiosulphate (2 g in 4 L of water) with 160 L of water at 80, 100, 120 and 140 gL⁻¹ salinity respectively. The population density in the containers was adjusted to a concentration of 1 org mL⁻¹ to avoid growth problems with respect container space [18].

Reproductive characteristics

When organisms reached sexual maturity and before seeing matings, were separated into male and females under the same culture conditions in 4 L containers for one week. In 200 mL glass container was introduced two males and one female (25 for each population) to determine reproductive characteristics: pre-reproductive, reproductive, post-reproductive period, number of broods, interval between broods, nauplii produced by female per broods, and cysts produced per female per broods [19, 20]. The same treatment was done for each salinity in the study. The dead males during experiment were replaced with males with active swimming [20]. Every day, until the female died, observations were made to determine the productive potential of each population, as well as salinity concentration.

Survival

The population survival was determined at the end of the experiment culture. The results (expressed as a percentage) were transformed with the formula:

$$\frac{\sqrt{(X\%)}}{100} + 0.5$$

Statistical analysis

To determine the average value and (\pm SD) at different variables, results were introduced into database in Microsoft Excel 2013 program. To obtain significant differences, between populations and experimental salinities, it was proceeded to perform a two-way variance analysis (ANOVA). When significant differences ($p < 0.05$) in the model was finding, multiple means comparison technique was applied by Tukey method. Data classification was based on grouping of populations according to their specific culture salinity [21, 22]. It was used SYSTAT 12.0 (Systat Software Inc., California, USA) program.

Results

The mean values (\pm SD) of organism's survival of different populations at experimental salinities are presented in Table 2. The ANOVA analysis did not indicate significant differences between populations when they were cultivated at same salinity, but when they are cultivated at different salinities, survival values shown significant differences ($p < 0.001$). The bifactorial analysis showed precisely that salinity variable has the greatest significance weight with 97.83%; population variable has only 0.096%; and the interaction of both variables did not present significant differences ($p = 0.527$).

Table 2: Mean values (\pm S.D.) of survival from each *A. franciscana* population at different tested salinities.

Populations	Experimental salinities (gL ⁻¹)			
	80	100	120	140
Pacific coastal waters				
JUCH	64	76	81	86
YAV	66	73	80	85
Inland waters				
SLP	65	75	78	84
TEX	63	75	79	84

The mean value (\pm D.S.) of pre-reproductive period are shown in Table 3. The ANOVA analysis showed no significant differences ($p > 0.05$) between populations when they were cultured at 80 gL⁻¹ salinity. In 100 gL⁻¹ of salinity, TEX presented significant differences ($p < 0.001$) with JUCH, SLP and YAV. At 120 gL⁻¹ of salinity, JUCH and YAV populations present significant differences ($p < 0.001$) with SLP and TEX. Regarding to the salinity of 140 gL⁻¹, the populations that present significant differences among

Table 5: Mean values (\pm S.D.) of post-reproductive period from each *A. franciscana* population tested at different experimental salinities.

Populations	Experimental salinities (gL ⁻¹)			
	80	100	120	140
Pacific coastal waters				
JUCH	7 \pm 2	8 \pm 1	10 \pm 1	15 \pm 2
YAV	8 \pm 1	8 \pm 2	10 \pm 3	14 \pm 1
Inland waters				
SLP	7 \pm 2	8 \pm 2	10 \pm 2	14 \pm 1
TEX	7 \pm 2	7 \pm 3	10 \pm 1	13 \pm 2

The mean values (\pm D.S.) of brood's quantity are shown in Table 6. The ANOVA analysis show that salinity of 80 gL⁻¹ was not significant different with respect JUCH/SLP ($p = 0.560$) and TEX/YAV ($p = 0.844$) populations. At 100 gL⁻¹ salinity, significant differences were observed with TEX

themselves ($p < 0.001$) are JUCH and SLP. The two-way ANOVA analysis showed that salinity variable had the highest significance with 67.32%; population variable with 5.17%; and the interaction of both variables with 8.22%.

Table 3: Mean values (\pm S.D.) of pre-reproductive period from each *A. franciscana* population tested at different experimental salinities

Populations	Experimental salinities (gL ⁻¹)			
	80	100	120	140
Pacific coastal waters				
JUCH	15 \pm 1	16 \pm 2	18 \pm 1	22 \pm 2
YAV	15 \pm 2	17 \pm 2	18 \pm 2	20 \pm 2
Inland waters				
SLP	15 \pm 3	17 \pm 1	16 \pm 1	19 \pm 2
TEX	12 \pm 2	14 \pm 1	16 \pm 1	19 \pm 3

The mean values (\pm D.S.) of reproductive period are shown in Table 4. The ANOVA analysis showed significant differences ($p = 0.046$) at 80 gL⁻¹ salinity between SLP with respect TEX and YAV populations; at 100 gL⁻¹ salinity, there were also significant differences ($p = 0.013$) between JUCH/SLP populations; at 120 gL⁻¹ of salinity, it was between SLP/TEX ($p = 0.047$) and at 140 gL⁻¹ of salinity, the population of JUCH and SLP with respect to the populations of TEX and YAV ($p = 0.001$). The bifactorial analysis showed that the salinity variable contributes to the significance with 58.93%, the population with 2.50% and the interaction of both variables with 9.15%.

Table 4: Mean values (\pm S.D.) of reproductive period from each *A. franciscana* population tested at different experimental salinities.

Populations	Experimental salinities (gL ⁻¹)			
	80	100	120	140
Pacific coastal waters				
JUCH	41 \pm 4	50 \pm 5	48 \pm 6	54 \pm 4
YAV	42 \pm 6	46 \pm 6	48 \pm 2	49 \pm 2
Inland waters				
SLP	44 \pm 4	45 \pm 1	51 \pm 6	54 \pm 4
TEX	41 \pm 4	48 \pm 3	47 \pm 2	51 \pm 2

Table 5 shows the mean values (\pm D.S.) of post-reproductive period. The analysis of ANOVA showed no significant differences ($p > 0.05$) in the salinities of 80, 100 and 120 gL⁻¹. Only at the salinity of 140 gL⁻¹, significant differences were observed ($p < 0.001$) in the JUCH population with respect to the other three populations. The two-way ANOVA analysis showed that salinity variable contributed 77.70% of significance; population variable with only 1.58%; and interaction of both variables the 2.61%.

population with respect other three populations ($p < 0.001$). At 120 gL⁻¹ salinity, there were no significant differences between JUCH/SLP ($p = 0.960$); and at 140 gL⁻¹ culture salinity YAV population with respect to other three presented significant differences ($p < 0.001$). The two-factor analysis

indicated that salinity contributes 62.21% of this significance; 15.19% to population variable; and interaction between both variables only 5.95%.

Table 6: Mean values (\pm S.D.) of number of broods per female from each *A. franciscana* population tested at different experimental salinities.

Populations	Experimental salinities (gL^{-1})			
	80	100	120	140
Pacific coastal waters				
JUCH	10 \pm 2	11 \pm 1	13 \pm 1	16
YAV	7 \pm 1	11 \pm 2	10 \pm 1	13 \pm 1
Inland waters				
SLP	11 \pm 2	12 \pm 1	13 \pm 1	16 \pm 2
TEX	8 \pm 2	9 \pm 2	12 \pm 2	15 \pm 2

Mean values (\pm D.S.) of interval between broods per female of each population were shown in Table 7. The ANOVA analysis showed significant differences in salinity of 80 gL^{-1} with the JUCH population with respect to TEX and YAV ($p=0.001$). The salinity of 100 gL^{-1} did not present significant differences ($p=0.947$). At 120 gL^{-1} of salinity there were no significant differences ($p=0.066$) and at 140 gL^{-1} the significant differences were between JUCH and YAV populations ($p=0.021$). The bifactorial analysis show that interaction of both variables has the 32.25% of total significance, unlike that of the population with 6.62%, and 3.77% of salinity variables.

Table 7: Mean values (\pm S.D.) of intervals between broods per female from each *A. franciscana* population tested at different experimental salinities.

Populations	Experimental salinities (gL^{-1})			
	80	100	120	140
Pacific coastal waters				
JUCH	3 \pm 1	5 \pm 1	3 \pm 1	3 \pm 2
YAV	4 \pm 1	3 \pm 1	3 \pm 1	4 \pm 1
Inland waters				
SLP	3 \pm 1	2 \pm 1	3 \pm 1	3
TEX	3 \pm 1	3 \pm 1	2 \pm 1	4 \pm 1

Nauplii per female mean values (\pm D.S.) are shown in Table 8. The ANOVA analysis show that 80 gL^{-1} culture salinity the populations that did not present significant differences among them were SLP and TEX ($p=0.686$). At 100 gL^{-1} salinity were the populations of JUCH/TEX ($p=0.996$) and SLP/YAV ($p=0.153$), which did not present significant differences. At 120 gL^{-1} salinity, TEX population did not show significant differences with JUCH and SLP ($p>0.05$), as well as SLP/YAV ($p=0.198$). At 140 gL^{-1} of salinity was YAV population that presented significant differences with JUCH, SLP and TEX ($p=0.001$). The two-ways ANOVA analysis showed that salinity variable contributed 54.45% of total significance; population variable with 25.64%; and interaction between both variables was 9.41%.

Table 8: Mean values (\pm S.D.) of nauplii produced per female from each *A. franciscana* population tested at different experimental salinities.

Populations	Experimental salinities (gL^{-1})			
	80	100	120	140
Pacific coastal waters				
JUCH	36 \pm 2	39 \pm 3	44 \pm 4	30 \pm 3
YAV	26 \pm 2	33 \pm 3	35 \pm 3	19 \pm 5
Inland waters				
SLP	29 \pm 1	32 \pm 3	39 \pm 3	29 \pm 2
TEX	27 \pm 3	39 \pm 3	41 \pm 8	28 \pm 4

Cysts per female mean values (\pm D.S.) are shown in Table 9. The production of cysts was given from the salinity of 100 gL^{-1} . The ANOVA analysis show significant differences ($p<0.001$) between populations cultivated in their respective salinity. However, at 100 gL^{-1} salinity JUCH population did not show differences with SLP ($p=0.191$) and YAV ($p=0.208$). At 120 gL^{-1} salinity there were no significant differences between JUCH/YAV ($p=0.994$) and SLP/TEX populations ($p=0.146$). At 140 gL^{-1} salinity only SLP/YAV did not shown significant differences ($p=0.428$). The bifactorial ANOVA analysis indicated that salinity variable contributed with 96.84% of total significance; population variable with 1.49%; and interaction of both variables with only 0.80%.

Table 9: Mean values (\pm S.D.) of cysts produced per female from each *A. franciscana* populations in their experimental salinities.

Populations	Experimental salinities (gL^{-1})			
	80	100	120	140
Pacific coastal waters				
JUCH	-	82 \pm 7	93 \pm 4	100 \pm 6
YAV	-	59 \pm 3	72 \pm 3	88 \pm 8
Inland waters				
SLP	-	72 \pm 4	79 \pm 4	92 \pm 6
TEX	-	66 \pm 2	73 \pm 5	84 \pm 7

Discussion

With respect to organism's survival to different salinities, very few aquatic organisms have the capacity to tolerate a wide range of salinities in their environment, for this it is necessary that they have a balance between the flow of ions from the external environment with their hemolymph through osmoregulation mechanisms that allow them an optimal regulation and therefore its survival. One of these organisms that can survive wide changes in salinity is the crustacean *Artemia*. Although in several studies the effect of salinity on this crustacean has been observed [23], only in the most recent ones, the influence of this factor mentioned [24, 25, 20, 26]. The response of Mexican populations of inland waters as well as coastal waters cultivated at different salinities below 60 gL^{-1} and above 120 gL^{-1} , the organisms die in the metanauplii stage because the osmoregulation mechanism is seen to be altered when organisms are placed directly at that concentration of salinity, while it is functional in the range of 60-120 gL^{-1} salinity, so it is likely that the large differences found are not due to the origin of the habitat of this crustacean, but is determined by the concentration of salt found in the environment [27]. In contrast to this [28] suggests that differences can be considered as responses to local biotopes and not only to intrapopulation responses to salinity, such as ploidy levels and the energy content of the nauplii larva.

When comparing the survival results with other works it can be mentioned that species and populations variables of different localities respond to a specific salinity. Differences registered with respect to salinity in different *Artemia* biotopes [29], provoke population isolation and therefore have caused different tolerance intervals between them. Those differences in survival [30], may also be due to different seasons, considering summer or winter species, which have totally different requirements and tolerances. As mentioned previously, salinity is the variable that affects studied Mexican populations and matches with what found by other authors [29], who mentions that this variable is that most affects survival of populations, because a low survival is observed in cultures at 35 gL^{-1} and increases when salinity

risers to 90 gL⁻¹. Other authors [25, 31], mentioned that when comparing with *A. franciscana* in salinities of 60 gL⁻¹ obtained survival of 24%, and observed that juvenile organisms grow slowly, and adults die in salinities below 38 gL⁻¹. However, was demonstrated with a population from Egypt that at salinity of 35 gL⁻¹ survival was greater than 60% [32]. Comparing the data with other *Artemia* species such as *A. salina*, *A. sinica*, *A. persimilis* and some parthenogenetic populations [25] indicate an interval of 0-24% survival in culture tests at 60 gL⁻¹ of salinity, [33] who studied *A. tibetiana* found 39% survival in cultures at 35 gL⁻¹ salinity [28], that studied parthenogenetic *Artemia* populations of Turkey and Greece, found 15% survival in cultures below 80 gL⁻¹ [20, 34, 35], and obtained 0% of survival in *A. urmiana* specie at 50 gL⁻¹ salinity. This low survival condition can have observed in Mexican population, because at salinities under 60 gL⁻¹, Mexican populations have 100% nauplii mortality, because they fail, in physiologically way, to activate the enzymes required for osmoregulation process [36]. The same process can be observed at salinities upper 140 gL⁻¹ culture salinity concentration in Mexican populations in agreement with authors [37]. Who works at salinities that exceeds 179 gL⁻¹, survival falls below 20%, because can be a damage in the irreversible osmoregulatory system due to concentration, frequency and duration of salinity in culture medium [36, 37].

In summary, salinity concentrations below 60 gL⁻¹ and above 120 gL⁻¹, affect the survival of *A. franciscana* populations, this agrees with the results obtained since it was observed that at a salinity of 80 gL⁻¹ the percentage of survival is 66%. However, this increase up to 85% when salinity reach 140 gL⁻¹. This may be due to response to salinity factor already was printed genetically in organisms, which allows them to better survive at salinities of 100-180 gL⁻¹ [38]; being therefore the salinity and not the origin of the population which makes the differences in survival significant. Obtaining this information allows a better management of the culture of *Artemia* populations under laboratory conditions when the salinity, temperature and food variables are known and maintained constantly.

Regarding the reproductive behavior studied, it can be mentioned that culture salinity concentration was the principally variable which modified it, but only in cysts production per female per broods, the origin of population plays a significant role together with salinity. In terms of Mexican populations grown under laboratory conditions, optimal conditions for cysts production are 100 and 120 gL⁻¹ [5]. This agrees with the present investigation since at those concentrations a greater number of cysts is observed. However, at a salinity of 140 gL⁻¹ the value continues to increase [19, 39]. Reproductive behavior can be influenced not only by salinity or population of origin, but also by the type of feeding and food concentration [19, 39]. In this work, the important variable to investigate was salinity, so the feeding and concentration variables remained constant throughout the experiment for all populations.

Maturity of population of *A. franciscana*, was achieved in less time when salinity is below 100 gL⁻¹ and increases with a salinity of 140 gL⁻¹ [20]. Authors such as [24, 25, 40] mention that to obtain cysts production in populations, salinity cannot be less than 80 gL⁻¹, in total agreement with this investigation with Mexican *A. franciscana*.

Oviparity occurred at salinity of 100, 120 and 140 gL⁻¹, although nauplii birth continued to occur. In the reproductive periods, the duration increased as salinity increased. The pre-reproductive period increased three days, the reproductive

period five days and the post-reproductive period two days in salinity of 140 gL⁻¹. As the reproductive period increases, populations can have more broods and consequently more nauplii or cysts. Different studies [25, 41] mentioned that optimal salinity of culture was that in which the duration of reproductive period of *Artemia* is greater.

The number of broods per female of Mexican populations cultured in laboratory was seven in salinity of 80 gL⁻¹ and 16 to 140 gL⁻¹. This reproductive characteristic is manifested with great variation as shown by other authors [19, 20, 41, 43], which reported for *A. franciscana* 4-14 broods at 90 gL⁻¹ salinity [19]; six broods per female in salinities of 70-80 gL⁻¹ [41], and two broods in 120 gL⁻¹ salinity [43]. In other *Artemia* species, such as *A. tunisiana* [19] they registered 4 to 7 broods per female at 90 gL⁻¹ salinity, and six broods with *A. urmiana* [20] at 50 gL⁻¹ salinity and one broods at 150 gL⁻¹ [20].

Regarding to the interval between broods, the value was two to five days for the salinity of 100 gL⁻¹ and three for the other salinities in experimentation. Other works [43] point out slight variations with the same species reported four days in 120 gL⁻¹ salinity culture concentration. While reported for *A. urmiana* [20] mentioned values of 4-5 days in salinity of 75-175 gL⁻¹. With parthenogenetic populations obtained values of 7 days at 120 gL⁻¹ of salinity. The fact is that one population have longer day duration between broods, allows better health recover of females and therefore have the capacity to carry out a greater number of broods and greater production [19].

With respect nauplii production per female at studied Mexican population, it was determined that lowest number was registered in crops at 140 gL⁻¹ and highest values in salinity of 120 gL⁻¹. In this reproductive characteristic a great variation in the information is also observed in different studies [19, 43] at a salinity of 120 gL⁻¹ with the same species.

The production of cysts in Mexican populations was observed in the range of 100, 120 and 140 gL⁻¹ culture salinities. This type of reproduction occurs when high salinity is maintained for prolonged periods [44, 43] with *A. franciscana*. On the other hand, the encysting process not only responds to the concentration of salt (osmotic pressure) and duration that organism remains in this culture medium, but also obeys to ionic composition of salt [45]. Variation in reproductive characteristics of different populations of *Artemia*, is given by the relationship that exists in salt concentration, and the amount of chlorides [46], because is necessary to find each one of optimal levels for its development and viability in their culture medium.

Conclusion

For everything described in this research, it can be assured that salinity plays a key role in survival and reproductive behavior of four Mexican populations studied and that these variables increase progressively until salinity of 120 gL⁻¹ with the exception to produce cysts, which continues to increase their number up to a salinity of 140 gL⁻¹. This generated knowledge, allows us to propose strategies for specie conservation, biomass and cysts production at laboratory level and transfer it to natural production systems, making an adequate production handling to supply the demand that this organism has in the aquaculture and mariculture industry.

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