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Reproductive potential of *Artemia franciscana* (Kellogg, 1906) from Mexico inland waters cultured in laboratory at different salinity

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Abstract

Four populations of *Artemia franciscana* from Mexico inland waters (Cuatro Ciénegas, Coahuila; Santo Domingo, Zacatecas; Las Salinas, San Luis Potosí and Texcoco, Edo. de México) were compared with respect to their reproductive potential. Each population was cultivated in 200 L beakers at a salinity of 60, 80, 100 and 120 gL⁻¹. Temperature was maintained at 25 ± 2 °C, with continuous light and aeration and pH between 8 to 10. The organisms were fed *ad libitum* with 50 mL of rice bran and 1 L of mixed solution of *Tetraselmis* sp. and *Pinnularia* sp. microalgae. When organisms reach sexual maturity stage, they were separated in 25 glass beakers of 250 mL, introducing one female and two males to determine reproductive potential in each salinity concentration experimental. Total life cycle was of 47- 68 days range. Reproductive period was of 30 to 45 days, number of broods were 7 to 11, nauplii produced per female were 39 to 58 and cyst production per female were 0 to 65. All values increase with salinity concentration. Calculated biomass was 161.28 to 501.12 g range and calculated cysts production was 100.46 to 180.40 g. This information could lay biological bases for laboratory culture exploitation or semi intensive culture system at their natural habitat, depending their culture management to obtain adult or cysts wet biomass.

Keywords: Artemia franciscana, Mexico, inland waters, reproductive potential, salinity

Introduction

One of the most important physicochemical variable which modify the organism's habitat is salinity, because it affects the life cycle and development of aquatic organisms directly and above all, those who lives in wide range salinity changes like coastal lagoons and water bodies or manmade salterns which produce salt. An organism that lives in this habitat is the brine shrimp *Artemia* who lives in those hypersaline habitats with substantial changes in salinity concentration [1, 2]. This brine shrimp is represented by distinct species which are widely distributed around the world, occupying coastal lagoons, inland waters bodies and water bodies used to produce salt [3, 4].

Artemia is a unique organism which has the capacity to adapt to habitats that have salinity concentration under 10 gL⁻¹ [5] and up to 340 gL⁻¹ [6]; and to habitats that have low biodiversity and relative simple food web structure ^[7]. This capacity of *Artemia*, allow to escape from their predators and food competitors, allowing with their nauplii-adult stage development be successful under these salinity extreme conditions. In some cases, allowing high densities due the presence of halobacterium and microalgae which resist same salinity conditions ^[8] and were used as food source for this non-selective efficiently filter feeding organisms ^[9].

In Mexico, it dominates the specie *Artemia franciscana* Kellogg 1906 ^[10], which is distributed at 26 sites both in coastal and inland saline waters, with specific salinity concentration and temperature ranges, so that each population may varied considerably regarding their physiological responses to cope specific physical-chemical conditions from each natural habitat. The carried-out studies with Mexican *Artemia* populations were about biometric and reproductive characteristics ^[11-25]. It is important to mentioned that *Artemia* production salinity concentration was between 40-60 gL⁻¹. Also, studies with reproduction and potential reproductive goal were made ^[25, 26].

Artemia was considered as an important economy source to fish and crustacean larviculture, because it was used as essential food to their development [27-29]. That is why in recent years, worldwide, the studies were indicted to find or to obtain a population Artemia strain that cover the potential characteristics (small size nauplii and cysts, rapid hatching, better development and, high adult and cysts biomass) that aquaculture needs.

The goal of this study was the comparison effects of different salinities concentrations (60, 80, 100 and 120 gL⁻¹) in reproductive potential (total days of life cycle, days of reproductive period, number of broods, nauplii and cysts production per female) of four Mexican inland waters *A. franciscana* populations to maintained populations at laboratory conditions. Not only obtained adult wet biomass was used as directly food to aquatic organisms, but also wet cysts biomass to maintain a cysts stock of Mexican

populations, allowing with this to maintain the biodiversity of this genus in Mexico.

Materials and methods

Cysts from Mexican A. franciscana

This study was made at Live Food Production Laboratory from El Hombre y su Ambiente Department from Universidad Autónoma Metropolitan de Xochimilco. The experimental cysts were taken from cysts bank storage at a fridge (-10 $^{\circ}$ C) to maintain dehydration and diapause process.

Geographical localization of studied A. franciscana populations

Local sites, abbreviation, habitat type and, geographical localization of experimental populations was shown in Table 1 and fig 1.

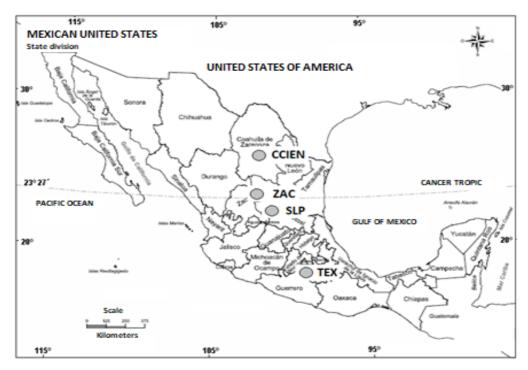


Fig 1: Geographical localization of Inland waters A. franciscana populations in México.

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

Locality	Abbreviation	Geographical coordinates
Cuatro Ciénegas, Coahuila	CCIEN	26°55'51''N; 102°02'22'' W
Santo Domingo, Zacatecas	ZAC	23°19'20''N; 101°46'51''W
Las Salinas, San Luis Potosí	SLP	22°39'16''N; 101°43' W
Ecatepec, Estado de México	TEX	19° 32' N; 99° 00' W

Organisms fed

During the experiments, the organisms were fed every third day with 50 mL of rice bran (300 g in 4 L of brine solution water) and one liter from each microalga: Tetraselmis sp. (Kylin: Butcher) and Pinnularia sp. (Cleve) at a concentration of 500 x 10^3 cel mL⁻¹) [30].

Experimental design

One gram from each *A. franciscana* population were set to hatch in a 4 L beaker with 40 gL⁻¹ salinity concentrations, pH of 8-10, temperature of 25 °C±2 °C, and permanent aeration and light ^[30]. Hatching nauplii were collected and placed in 200 L beakers and filled with 160 L of 60, 80, 100 and 120 gL⁻¹ salinity water concentration. Population density was

adjusted to 1-2 org mL⁻¹ to avoid growth problems because available space habitat ^[30].

For each experimental treatment, when organisms reached sexual maturity were separated by sex (male/female), and cultured at same physicochemical conditions for two weeks. After that time, organisms were placed in 25 glass beakers of 250 mL (two males/one females per beaker) to determined daily couple match, total days of life cycle, reproductive period (days), number of broods and, nauplii and cysts production per female. The dead males were replaced [31]. Every day, until females died, observations were made to determine reproductive potential from each salinity concentration, which was monitored with an AO (0-150 gL⁻¹ range) refractometer to maintain salt concentration.

Information processing

All obtained data was placed in a data base in Excel 10 program to determined mean values (±S.D.). To determined reproductive potential from each experimental salinity concentration, values of nauplii and cysts produced per female were multiplied by brood's number to determined total production per female and values were extrapolated to 160 L volume water production, considering a 1 org mL⁻¹ density, with a percentage of 50% of females and survival of 30%.

To determined total wet biomass of nauplii and cysts, their total production values were multiplied per 0.001 g for adult stage biomass and per 0.000001 g for cysts stage (mean values obtained per 100 adult stage organisms and 1,000 cysts), using a Digital Granary Scale Balance Ohaus (0.0001 g accuracy).

The total number of broods, nauplii and cysts produced, were normalizer with following formula:

Transformed data = $\sqrt{\text{Value} + 0.5}$

Statistical analysis

To determine significant differences (p<0.05) between salinities concentration and reproductive variables in all populations, it was applied a two-ways ANOVA analysis and Tukey test to compare multiple mean values, considering salinity and population as principally variables [32, 33], using Systat 12.0 (Systat Software Inc., California, USA) program.

Results

Total life cycle

In Table 2 it is shown the mean values of total life cycle per female from each experimental salinity concentrations (60, 80, 100 and 120 gL⁻¹). It can be observed that mean values increase with salinity. ZAC population showed the lowest value (47±4 days) at 60 gL⁻¹ salinity and higher values was shown in CCIEN population (68±3 days) at 120 gL⁻¹ salinity. ANOVA analysis show that 60, 80, 100 and 120 gL⁻¹ experimental salinities did not present significant differences (p=0.690, p=0.414, p=0.353, p=0.633 respectively) between populations, but with respect to same population cultured at different salinities, it did show significant differences (*p*<0.001) in all populations.

Two-ways ANOVA analysis show significant differences (p<0.001). Salinity has 81.59% of that significance weight, population variable only has 0.67% of that difference and interaction between salinity/population the 0.44%.

Table 2: Mean values (\pm S.D.) of life cycle (days) of Inland waters *A. franciscana* populations cultured at experimental salinities.

Donulation	Experimental salinity (gL ⁻¹)				
Population	60 80		100	120	
CCIEN	50 ±5	57 ±3	62 ±2	68 ±3	
ZAC	47 ±4	55 ±4	61 ±4	67 ±3	
SLP	48 ±4	56 ±3	63 ±3	67 ±2	
TEX	49 ±3	54 ±4	61 ±3	67 ±3	

Reproductive period

Table 3 show the mean values of reproductive period (in days) at different experimental salinities. The lowest value was shown in TEX population with 28 ± 6 days at $60~{\rm gL^{-1}}$. Highest value was shown in CCIEN population with 45 ± 7 days at $120~{\rm gL^{-1}}$.

Table 3: Mean values (±S.D.) of reproductive period duration (days) of Inland waters *A. franciscana* populations cultured at experimental salinities.

Donulation	Experimental salinities (gL ⁻¹)				
Population	60 80		100	120	
CCIEN	33±10	39 ±5	35 ±7	45 ±7	
ZAC	30 ±9	37 ±8	38 ±6	43 ±6	
SLP	31 ±6	37 ±9	40 ±7	40 ±5	
TEX	28 ±6	36 ±8	44 ±7	41 ±4	

ANOVA analysis shown no significant differences between 60, 80 and 120 gL⁻¹ (p=0.663, p=0.786, p=0.171 respectively) salinities, but at 100 gL⁻¹ experimental salinity, CCIEN/TEX populations showed significant differences (p=0.21). With respect to obtained values from each population cultured at different salinities, ANOVA analysis showed significant differences between all populations (CCIEN p=0.002; ZAC p=0.004; SLP p=0.027; TEX p<0.001). Applied Tukey test was found that in CCIEN population, the significant differences were shown at 120/60 and 120/100 (p=0.002; p=0.011 respectively); to ZAC and SLP populations at 120/60 (p=0.002, p=0.034 respectively) salinities and for TEX population at 60/80 (P=0.049) and 100/120 gL⁻¹ (p<0.001) salinities.

Two-ways ANOVA analysis shown significant differences (p<0.001). Salinity variable has 27.53% of that significance, population variable has 0.19% and for their interaction (salinity/population) was 6.89%.

Number of Broods

Table 4 show mean values of number of broods at experimental salinities. The lowest value founded in all population was at 60 gL^{-1} salinity. Meanwhile, highest values were found in CCIEN population with 12 ± 3 days at 120 gL^{-1} . ANOVA analysis showed no significant differences between all salinities ($60 \text{ gL}^{-1} \text{ p=}0.114$; $80 \text{ gL}^{-1} \text{ p=}0.179$, SLP p=0.513; TEX p=0.597). With respect to each population cultured at differences in all cultured populations (p<0.001). However, Tukey test showed that to CCIEN population there were significant differences (p<0.001) between 60/100, 120 and 80/60, 120 salinities. For ZAC population, were 60/80, 100, 120 (p<0.001), for SLP population were 60/120 salinities (p<0.001) and, for TEX population 100/60, 80 and 120/60, 80 salinities (p<0.001).

The two-ways ANOVA analysis showed significant differences (p<0.001). Salinity variable had the highest weight value of that significance with 49.21%, population variable with 0.94% and for their interaction (salinity/population) was 3.00%.

Table 4: Mean values (±S.D.) of number of broods of Inland waters *A. franciscana* populations cultured at experimental salinities.

Populations	Experimental salinities (gL ⁻¹)				
Populations	60	80	100	120	
CCIEN	7 ±1	9 ±1	11 ±2	12 ±3	
ZAC	7 ±1	9 ±2	11 ±3	11 ±2	
SLP	7 ±1	9 ±1	9 ±2	11 ±2	
TEX	7 ±1	8 ±1	10 ±2	11 ±1	

Nauplii produced per female

Table 5 show mean values of nauplii produced per female at experimental salinities. The lowest value was obtained by TEX population with 32±10 at 60 gL⁻¹, meanwhile highest

mean value was from CCIEN and ZAC populations with 58 ± 6 and 58 ± 9 respectively at $120~{\rm gL^{-1}}$.

Table 5: Mean values (±S.D.) of nauplii produced per female of Inland waters *A. franciscana* populations cultured at experimental salinities.

Populations	Experimental salinities (gL ⁻¹)				
Populations	60	80	100	120	
CCIEN	39±10	46 ±5	48 ±7	58 ±6	
ZAC	39 ±11	50 ±11	53 ±10	58 ±9	
SLP	46 ±10	47 ±6	53 ±8	57 ±8	
TEX	32 ±10	45 ±5	51 ±8	57 ±9	

ANOVA analysis showed no significant differences in all experimental salinities (60 gL⁻¹ p=0.052; 80 gL⁻¹ p=0.588, 100 gL⁻¹= 0.424; 120 gL⁻¹= 0.969). Regarding to obtained mean values at each population cultured at different salinity concentration, ANOVA analysis showed significant differences between all populations (p<0.001). However, Tukey test showed to CCIEN population significant differences at 120/60, 80, 100 salinities (p<0.001). For ZAC population between salinities 60/100,120 (p<0.001), for SLP population at 60/120 (p=0.022) salinity and TEX population at 60/80,100,120 and 120/80 salinities (p<0.001).

Two-ways ANOVA analysis showed significant differences (p<0.001). Salinity variable had the highest weight value of significance with 37.78%, population variable with only 2.64% and the interaction between salinity/population with 3.73% of significant weight.

Cysts produced by female

Table 6 show mean values of cysts produced per female in all experimental salinities. Salinities of 60 and 80 gL $^{-1}$ did not shown cysts production in all populations. The lowest value was 54 ± 5 with CCIEN population at 100 gL $^{-1}$. The highest mean value was for TEX population with 65 ± 3 at 120 gL $^{-1}$ and 65 ± 4 for SLP population at 100 gL $^{-1}$ salinity concentrations.

Table 6: Mean values (±S.D.) of cysts production per female of Inland waters *A. franciscana* populations cultured at experimental salinities.

Populations	Experimental salinities (gL ⁻¹)				
	60	80	100	120	
CCIEN	0	0	54±5	60 ±6	
ZAC	0	0	57 ±5	60 ±5	
SLP	0	0	65 ±4	63 ±7	
TEX	0	0	57 ±7	65 ±3	

ANOVA analysis did not show significant differences for 100 gL⁻¹ salinity (p=0.001), but Tukey test showed that SLP population have significant differences with CCIEN, ZAC and TEX (p<0.001). At 120 gL⁻¹ salinity, did not show significant differences between populations (p=0.130). With respect values obtained at same population but cultured at different salinities, ANOVA analysis shown significant differences (p<0.001). However, Tukey test show to CCIEN no significant differences (p=1.000), for ZAC population to 100/120 (p=0.319) and 60/80 (p=1.000), for SLP population did not show significant differences at 100/120 (p=0.746) and 60/80 (p=1.000). TEX population did not show significant differences at 60/80 salinities (p=1.000).

The two-ways ANOVA shown significant differences between variables (P<0.001). The highest weight significance was for population variable (97%). For salinity variable was only 0.18% and for the interaction between salinity/population was 0.36%.

Reproductive potential

Table 7 show theoretical reproductive potential values in all experimental salinities. The mean values increase with salinity concentration in all populations. The reproductive potential increase from 161.28 g (60 L⁻¹, TEX), to 501.12 g (120 gL⁻¹, CCIEN). Cysts biomass increase from 104.65 g (100 gL⁻¹, TEX), to 180.40 g (120 gL⁻¹, CCIEN) in 21 days of cultured system. The Mexican *A, franciscana* inland waters populations began to produce cysts at 100 gL⁻¹ salinity concentrations.

Table 7: Theoretical values of reproductive potential of Inland waters A. franciscana cultured at different experimental salinities.

Donalotions	Culture salinity	Culture beaker: 160 L. Density 1 org mL ⁻¹ . Survival: 30%.					
Populations		Total nauplii	Calculated biomass (g)	Total cysts	Calculated cysts biomass (g)		
CCIEN	60	6,552,000	196.56	0	0		
	80	9,936,000	298.08	0	0		
	100	12,672,000	380.16	1,129,075,200	112.90		
	120	16,704,000	501.12	1,804,032,000	180.40		
ZAC	60	6,552,000	196.56	0	0		
	80	10,800,000	324	0	0		
	100	13,992,000	419.76	1,315,947,600	131.59		
	120	15,312,000	459.36	1,515,888,000	151.58		
SLP	60	7,728,000	231.84	0	0		
	80	10,152,000	304.56	0	0		
	100	11,448,000	343.44	1,004,562,000	100.45		
	120	15,048,000	451.44	1,564,239,600	156.42		
TEX	60	5,376,000	161.28	0	0		
	80	8,640,000	259.20	0	0		
	100	12,240,000	367.20	1,046,520,000	104.65		
	120	15,048,000	451.44	1,613,898,000	161.38		

Discussion

To make a correct management of inland waters A. franciscana populations from Mexico, it is important to know that not only food type variable can modify adult stage or cysts biomass, it should also be considered food quality that was applied in A. franciscana culture and it is important to apply mixed diets that contain microalgae and bacteria that allow better growth and maturity female rates and get better nauplii or cysts production [33]. Also, it is important to considered food concentration in culture medium, since this depends nauplii survival and growth until they reach maturity stage and their future reproductive period as adult stage or cysts biomass. One variable which was not considered to make proper Artemia sp. culture in laboratory conditions was to know sex proportion in culture medium, because even though it is desirable to have higher female concentration, it is better when male proportion is higher to assure females fertilization to obtain higher nauplii or cysts production [34].

Carbohydrates intake in *Artemia* sp. diet is important to obtain maximum total length and highest values of biomass production. At this aspect, not only external carbohydrates source was important but also need to considered bacteria source from culture medium. These two conditions contribute to nutrient rupture from microalgae source and can be better used because of their transformation by produced bacteria enzymes [35].

Many times, when salinity increase in culture medium, it is not considered density or viscosity increase in culture medium, this condition increase organisms energetic budget to maintain actively swimming, not only to obtain their food, but also oxygen intake to make their physiological functions and in this case, carbohydrates plays an important role. This energetic budget, not considered, can delay growth in cultured organisms to reach adult stage and cause a decrease in oviparous or ovoviviparous reproduction. That is why is important to consider a carbohydrates input with rice or wheat bran or bacteria source. Bacteria source can be obtained by Biofloc system, which is rich of heterotrophic bacteria [35].

It is important to increase a carbon/nitrogen supplement when *Artemia* sp. culture is made in high densities, because it promotes growth, organism's maturation and increase the fecundity values ^[36]. These authors used pork and tapioca composed to increase C/N relation and they observed that cysts production increased 2.8 kg (control test) to 9.96 kg (wet weight ha⁻¹). Incorporation of adequate fertilizer source, improve not only microalgae production, but also the growth of heterotrophic bacteria; this extra contribution of energy source, allow that biological activities under adverse physical-chemical conditions, can be carried out properly. This C/N relation (20:1), can increase cysts production per female in 24 to 90 (120 m² pond), and cysts production was 28 to 38 kg (wet weight) ^[37]. If obtained information in this study is extrapolated to that area, it would be obtained a cysts production of 5.00 to 8.62 kg month⁻¹.

Also, it is important to considered nauplii density inoculation in culture medium ^[38]. When 3 to 5 million of nauplii m² were inoculated, biomass (adult stage) production was 5 kg 1,000 m² day⁻¹ and cyst production of 2 kg 1,000 m² month⁻¹. Unlike this study results, when were extrapolated to that area, was obtained 2.14 to 6.65 kg day⁻¹ (biomass) and 41.69 to 71.87 kg month⁻¹ of cysts.

It is important to mention that obtained results in reproductive potential studies (biomass and cysts production), cannot agree with reproductive potential estimated in specific habitat or physicochemical condition, because can be influenced for multi variable conditions, and *Artemia* sp. populations replay different from each variable like salinity, oxygen concentration, organism densities, type of food and density applied ^[9]. Their results obtained 34 cysts female⁻¹ at 80 gL⁻¹ salinity and 15 to 127 cysts female⁻¹ at 120 gL⁻¹, unlike findings in this study where at 80 gL⁻¹ salinity, populations did not produce cysts, meanwhile at 100 and 120 gL⁻¹ salinities, quantity were 54-65 cysts female⁻¹.

Another important variable to produce cysts was photoperiod, mostly when culture medium has most hours in darkness at >25 °C ^[39]. Lowest temperature culture conditions, with continuous light decrease cysts production and stimulate ovoviviparous condition, that is why in this study nauplii production was maintained during all experiment, almost at 100 and 120 gL⁻¹ salinities. Food and oxygen levels were not critical variables to determined oviparous condition, but they are important for growth and reproduction of *Artemia* sp. populations ^[39].

Nauplii and cysts production are related with light intensity [40]. Reproduction pattern can be modified by light type and their intensity. These authors mentioned that at low light intensity, oviparous condition increase in culture medium (57.92% for 0 luxe; 22.65% 5000 luxe). But with respect to their study with female organisms of *A. urmiana*, they observed that number of offspring oscillate between 935 nauplii and 685 cysts up 100 luxe, meanwhile below that intensity it decrease to 234 nauplii and 217 cysts. These authors also mentioned that when *Artemia* sp. culture medium was exposed to 2000 to 5000 luxe increase nauplii and cysts production because population organisms showed higher swimming activity and higher gregarious behavior, which induced to reproduction activity.

In other studies ^[41, 42], were mentioned that cysts production began at salinity up to 95 gL⁻¹, according with this experiment, because below that salt concentration, all population did not produce cysts. At same time, it is important to know that pond or beaker depth induce oviparous condition in *Artemia* sp. populations, because cysts production decrease below 40 cm depth.

Artemia biomass production of 15.72 gL⁻¹ can obtained after 15 culture days, when 10 nauplii mL⁻¹ in 1.5 L beakers were inoculated ^[43], unlike with was obtained in this study with 161.28-501.12 g (160L⁻¹). Regarding to the obtained in culture beakers of 700 L ^[44] with 26.45 to 33.86 g of cysts and 813.6 a 1226.7 g of biomass in 38 culture days. In this study were obtained 705.6 to 2,192 g of biomass in 21 culture days (values extrapolated to 700 L). When A. franciscana specie was cultured at 100 gL⁻¹ ^[45], it was obtained 162 cysts per 40 females, at 120 gL⁻¹ 196 cysts per 40 females and in 140 gL⁻¹ salinity culture medium there are no data, because population died. Unlike this study, that female organisms produce 2,160 (100 gL⁻¹) to 2,600 (120 gL⁻¹) per 40 females.

Finally, biological implications for these results allow to make better management of natural habitat *Artemia* sp. populations, not only for specie conservation, but also for their commercial exploitation management, maybe not complete, biomass and cysts demand to aquaculture or aquarist industries in Mexico, but must be able to supply at local state country level.

Conclusion

These results show that is possible to make laboratory management of A. franciscana Mexican populations to obtain

cyst and adult stage biomass production, which can be used as food source to fish and crustacean growth, reproduction, or survival experiments.

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