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Salinity effect on reproductive potential of four *Artemia franciscana* (Kellogg, 1906) Mexican populations grown in laboratory

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Abstract
Potential production of four Mexican populations of *A. franciscana* were obtained in laboratory at different salinities (80, 100, 120 and 140 gL^{-1}) culture medium in 200 L beakers at 25 ± 2 °C, with constant light and aeration and pH 8-10. Strains were fed with 50 mL of suspension rice bran and 1 L of *Tetraselmis* sp. and *Pinnularia* sp. microalgae culture. When populations reached sexual maturity, they were separated into 25 vials of 250 mL (one female and two males) to determine nauplii and cysts production. Broods produce were 7-11/13-16, nauplii 46-54; 57-66 and cysts 52-65; 67-85 by female increased with salinity. Nauplii production decreases at 140 gL^{-1} salinity (38-45 per female). The productions obtained were 273-411 g biomass in 120 gL^{-1} salinity and 4-7 g of cysts in 140 gL^{-1} salinity. This information can allow potential productions of these organisms in semi intensive culture ponds in their own natural habitats.

Keywords: A. franciscana, salinities, cultivation, rice bran, microalgae.

1. Introduction
Salinity is one of the most important physico-chemical variables in the environment because it directly affects development and life cycle of organisms; this is particularly true in aquatic environments and much more noticeable in habitats that have wide fluctuations in salt concentrations, such as coastal lagoons and salt ponds. Among the organisms that inhabit such environments are found crustaceans belonging to the *Artemia* genus, living in hypersaline systems [1, 2].

The genus *Artemia* comprises a number of species that are widely distributed in the world, occupying from inland bodies of water to coastal lagoons and coastal water bodies engaged in salt production [3, 4]. *Artemia*, which has the ability to adapt to changing environmental conditions with respect to salinity that can range from concentrations under 10 gL^{-1} [5] up to 340 gL^{-1} [6] habitats with low biodiversity and a relatively simple trophic structure [7]. This "brine shrimp" as it is known, is favored by such conditions due to absence of predators and competitors for food, thereby allowing development (nauplio-adult) to be successful under these extreme conditions of salinity, in some cases reaching high densities due to presence of halobacteria and microalgae that resist the same adverse conditions [8] and serve as a food source for this efficient filtering non-selective organism [9].

Information about survival, growth, biometry, reproductive characteristics and life cycle, as well as specific responses (food, salinity and temperature) of bisexual and parthenogenetic Artemia species were collected around the world [2, 3, 5, 10, 21]. Many of these investigations have contributed to the assessment of genetic and environmental components, as well as the comparison of life cycle characteristics and different reproductive strategies (ovoviviparous vs oviparous) among different populations [16, 17, 21, 23].

Artemia was considered as economic important source in fish and crustaceans larviculture, because it is essential food for their development The *Artemia* genus is considered an economically important resource in fish and marine crustacean's larviculture, since it is a suitable food for their development. [18, 24, 25]. That is why in recent years, worldwide research has been directed to discover or obtain an *Artemia* population which covers the potential features (cyst and small nauplio; low hatching rate, good development and high biomass or

Cysts productions) to be used in this industry of aquaculture.
Artemia franciscana Kellogg 1906 is the principal specie [26], which is distributed at 26 habitats in coastal bodies of salt water and inland water with different salinity conditions and specific temperature, so each populations vary considerably from their physiological response to meet the conditions prevailing in their different aquatic environments. Some studies with Mexican *Artemia* populations about biometric and reproductive characteristics were made [27-37]. It is important to show that salinity range used in the production of Mexican *Artemia* was 40-60 gL^{-1}.
The mean goal of this study was to compare the salinity effect (80, 100, 120 and 140 gL^{-1}) on the reproductive potential (nauplii and cysts produced per female) of four Mexican populations of *A. franciscana* located in Pacific coastal waters and inland waters. These information can allow to maintain at laboratory conditions, the Mexican *Artemia* "stock" (cysts), thereby conserving the biodiversity of this specie in Mexico and also can estimate the field yield live biomass and cysts production in natural habitats.

2. Materials and Methods

2.1. Mexican *A. franciscana* cysts

This study was made at Live Food Production Laboratory, El Hombre y su Ambiente Department of Universidad Autonoma Metropolitana Xochimilco. The cyst were obtained from a cysts bank that was stored in refrigerator conditions (-10 °C) to maintain their dehydration (under 10% humidity) and diapause conditions.

2.2 Geographical localization of Mexican *Artemia* populations

The locality zone, abbreviation, habitat type and geographical localization are shown at Table 1 and Fig. 1.

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

Locality zone	Geographical coordinates
Pacific coastal waters	
Juchitan, Oaxaca	16° 26' N; 95° 01' W
Yavaros, Sonora	26°41' N; 109°31' W
Inland waters	
San Luis Potosi, S.L.P.	22°38' N; 101°43' W
Texcoco, Estado de Mexico	19° 32' N; 99° 00' W

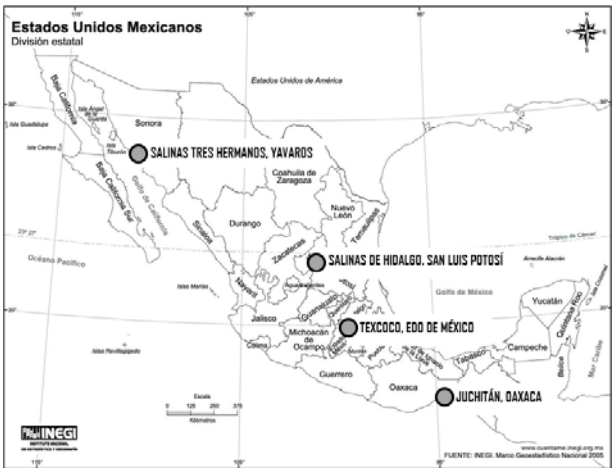

Fig 1: Geographical localization of Mexican *A. franciscana* populations at coastal and inland waters.

2.3 Food production

During the experiment the organisms were fed every third day with 50 mL of rice bran (300 g 4 L^{-1} of 100 gL^{-1} salinity water) and one liter of microalgae's *Tetraselmis* sp. (Kylin) Butcher and *Pinnularia* sp. Cleve at 500 x 10^3 cells mL^{-1} concentration) [38].

2.4 Experimental design

The cysts (1 g per each population), were hatching in 4 L beakers with three liters of 40 gL^{-1} saline water, with pH 8-10; 25 ± 2 ºC temperature, constant light and aeration [38]. The hatching nauplii were collected and transferred in four 200 L beakers with 160 L of 80, 100, 120 and 140 gL^{-1} respectively. The total density in beakers were adjusted to 1 organism mL^{-1} to avoid grow problems for space availability at culture medium [38]. When organisms reach sexual maturity they were separated by sex and cultured at same physiochemical conditions in 4 L beakers. From each population and salinity test, one female and two males were introduced in 25 glass beakers (250 mL capacity) at same culture conditions to determine mating, broods number, nauplii and cysts per female. The males were replaced when they die [21, 39]. Every day, observation and counting were made to determine reproductive potential from each *Artemia* population at different salinity tests until female die.

2.5 Salinity monitoring

Every day, the salinity concentration was monitoring with AO refractometer (0-150 gL^{-1}) to maintained salinity test concentration.

2.6 Statistical

From each population it was obtained mean values (± S.D.) of number of broods, brood interval, nauplii and cysts produced per female. To determine significant differences (P<0.05), two ways ANOVA test was made. When significant differences were found, multiple mean compare values were tested by Tukey technique. Salinity and population were considering as test variables [40]. To assure normality of values a Box Plot and Leaf and Stem tests were made with Systat 12.0 (Systat Software Inc., California, USA) program [40-41].

2.7 Reproductive potential

To determine the reproductive potential from each population at different salinity concentrations tests, the mean values of nauplii and cysts per female were multiplied by number of broods. These values were extrapolated to 160 L culture beakers with 1 organism mL^{-1} density, considering 50% only for females and 60% of survival. Values of nauplii and cysts obtained were multiplied by 0.01 g from each adult to obtain live biomass and 0.0001 g from each cysts to obtain total cysts production. To obtain these values, 100 adult organisms and 100 cysts were weighed with a digital OHAUS balance with 0.0001 g precision.

3. Results

Table 2 shows the mean values of number of broods per female at different experimental salinities (80, 100, 120 and 140 gL^{-1}). The mean value increase with salinity. Yavaros *Artemia* population showed the lowest values with 7-13 broods per female and highest in San Luis Potosi populations with 11-15 broods per female.
ANOVA analysis shows no significant differences in Yavaros population between 100 and 120 gL^{-1} salinity tests (P=0.875);

to Juchitan population in 100 gL^{-1} salinity with respect 80 gL^{-1} test (P=0.098) and with 120 gL^{-1} salinity test (P=0.698). With respect to San Luis Potosi population at 100 gL^{-1} salinity test, it did not show significant differences with 80 gL^{-1} (P=0.12) and 120 gL^{-1} (P=0.861), and Texcoco population at 100 gL^{-1} salinity test did not show significant differences with 80 gL^{-1} (P=0.627) experimental salinity. The two way ANOVA test show significant differences (P<0.05) with population, salinity experiment and both variables with a significant percentage of 15.87, 63.09 y 4.12% respectively.

Table 2: Mean values (±S.D.) of number of broods per female from each *A. franciscana* population at different tested salinities.

Populations	Experimental salinities			
	80 gL^{-1}	100 gL^{-1}	120 gL^{-1}	140 gL^{-1}
Yavaros	7 ±1	11 ±2	10 ±1	13 ±1
Juchitan	10 ±2	11 ±1	13 ±1	16 ±2
San Luis Potosi	11 ±2	12 ±1	13 ±1	15 ±2
Texcoco	8 ±2	9 ±2	12 ±2	15 ±2

At Table 3, mean values of nauplii produced per female are shown at different experimental salinities. In all tested populations, mean value of nauplii quantity increases with until it reaches 120 gL^{-1} salinity concentration, while at 140 gL^{-1} the mean value declines in all. Yavaros population showed the lowest values (38-57 nauplii per female) and Juchitan population the highest with 40-66 nauplii per female. The ANOVA test shown that Yavaros population did not show significant differences between salinities 100/120 gL^{-1} (*P*=0.640) and between 80/140 gL^{-1} salinities (*P*=0.153). With respect Juchitan population, the nauplii values produced at 100 gL-1 salinity did not show significant differences with 120 gL^{-1} test (*P*=0.619) and 80 gL^{-1} salinity test (*P*=0.062). San Luis Potosi and Texcoco populations shown significant differences between all salinities tests (*P*<0.001). Two ways ANOVA test show significant differences between population and salinity variables (5.95 y 78.52% respectively), but not between their interaction (P=0.735) with only 0.021% of significance.

Table 3: Mean values (±S.D.) of nauplii produced per female from each *A. franciscana* population tested at different experimental salinities.

Populations	Experimental salinities			
	80 gL^{-1}	100 gL^{-1}	120 gL^{-1}	140 gL^{-1}
Yavaros	46 ±3	54 ±9	57 ±13	38 ±2
Juchitan	54 ±2	61 ±6	66 ±9	40 ±6
San Luis Potosi	52 ±2	59 ±6	65 ±6	45 ±4
Texcoco	52 ±2	59 ±4	65 ±6	42 ±4

Table 4 shows mean values of produced cysts per female at different experimental salinities. At 80 gL^{-1} salinity test did not show cysts production in all populations. In all the other tested salinities, the cysts production increase with salinity. Yavaros population showed lowest values with 52-67 cysts produced per female and Juchitan population show the highest values with 65-85 cysts produced per female.

The one way ANOVA test did not show significant differences between Yavaros and Juchitan populations with respect cysts productions at 100 and 120 gL^{-1} salinities (P=1.000; P=0.992 respectively). For San Luis Potosi population, 100, 120 and 140 gL^{-1} salinity tests shown significant differences between them (P<0.001), meanwhile Texcoco strain did not show significant differences between 120 and 140 gL^{-1} salinity tests. The two-way ANOVA indicates significant differences between population and salinity variables and their interaction with 1.50, 96.11 and 1.11% of significance respectively.

Table 4: Mean values (±S.D.) of cysts produced per female from each *A. franciscana* populations in their experimental salinities.

Population	Experimental salinities			
	80 gL^{-1}	100 gL^{-1}	120 gL^{-1}	140 gL^{-1}
Yavaros	0	52 ±6	55 ±8	67 ±4
Juchitan	0	65 ±7	62 ±9	85 ±8
San Luis Potosi	0	59 ±4	66 ±4	78 ±4
Texcoco	0	55 ±6	64 ±10	71 ±7

At Table 5-8 are shown theoretical values of reproductive potential from these four Mexican *A. franciscana* populations at different experimental salinities. The reproductive potential increases with salinity. The biomass production increases from 154.56 g 160 L^{-1} (with density of 1 organism mL^{-1}) to 411.84 g. The cysts production per gram increases from 0 to 6.53 g (21 culture days). The Mexican *A. franciscana* population that showed lowest production is Yavaros at 80 gL^{-1} salinity culture medium, meanwhile Juchitan population show highest production at 120 gL^{-1} salinity test. These four Mexican *A. franciscana* population began to produce cysts at 100 gL^{-1} salinity culture medium. Texcoco show the lowest values at 100 gL^{-1} with only 2.38 g produced and the highest Juchitan population with 6.53 g produced at 140 gL^{-1} salinity culture medium.

Table 5: Theoretical values of reproductive potential of four Mexican *A. franciscana* tested populations, cultured at 80 gL^{-1} salinity concentration.

Population	Number of broods	Nauplii per female	Cysts per female	Total nauplii produced per female	Total cysts produced per female	Live biomass (g)	Cysts biomass (g)
Yavaros	7	46	0	322	0	154.56	0
Juchitan	10	54	0	540	0	259.20	0
San Luis Potosi	11	52	0	572	0	274.56	0
Texcoco	8	52	0	416	0	199.68	0

Table 6: Theoretical values of reproductive potential of four Mexican *A. franciscana* tested populations, cultured at 100 gL^{-1} salinity concentration.

Population	Number of broods	Nauplii per female	Cysts per female	Total nauplii produced per female	Total cysts produced per female	Live biomass (g)	Cysts biomass (g)
Yavaros	11	54	52	594	572	285.12	2.75
Juchitan	11	61	65	671	715	322.08	3.43
San Luis Potosi	12	59	59	708	708	339.84	3.40
Texcoco	9	59	55	531	495	254.88	2.38

Table 7: Theoretical values of reproductive potential of four Mexican *A. franciscana* tested populations, cultured at 120 gL^{-1} salinity concentration.

Population	Number of broods	Nauplii per female	Cysts per female	Total nauplii produced per female	Total cysts produced per female	Live biomass (g)	Cysts biomass (g)
Yavaros	10	57	55	570	550	273.60	2.64
Juchitan	13	66	62	858	806	411.84	3.87
San Luis Potosi	13	65	66	845	858	405.60	4.12
Texcoco	12	65	64	780	768	374.40	3.69

Table 8: Theoretical values of reproductive potential of four Mexican *A. franciscana* tested populations, cultured at 140 gL^{-1} salinity concentration.

Population	Number of broods	Nauplii per female	Cysts per female	Total nauplii produced per female	Total cysts produced per female	Live biomass (g)	Cysts biomass (g)
Yavaros	13	38	67	494	871	237.12	4.18
Juchitan	16	40	85	640	1360	307.20	6.53
San Luis Potosi	15	45	78	675	1170	324.00	5.62
Texcoco	15	42	71	630	1065	302.40	5.11

4. Discussion

One of the principal variables which modified the *Artemia* culture management to produce live biomass or cysts is available food in optimal concentration and their nutritional quality. That is why it is important to supply mixed diets with microalgae, bacteria or some protozoa organisms which allow best grow and maturity female rates to obtain better nauplii or cysts productions [42]. Is also important to consider the food abundance, since nauplii survival and consequently the cysts production depends on it. An increase in *Artemia* sp. biomass in culture medium, allows a better quantity of cysts production greater amount of cysts, but it is important to consider the relation sex ratio, because greater presence of females allow an increase in the number of produced cysts [43]. Different authors mentioned the important contribution value of carbohydrates in diet supply in *Artemia* sp. to obtain maximum total length and biomass amount [44]. Not only carbohydrates are an important source of energy to organisms, bacteria source in culture medium can contributed too to this energy content. The carbohydrates or bacteria presence in *Artemia* diet contribute to nutrient breakdown from microalgae due to their enzymes content. Many times when salinity increase in culture medium, it must be considered the density or viscidity of water, because of the energy cost to active swimming in this culture medium by organisms not only to obtain their food but to obtain the necessary oxygen to make their metabolism correctly. This energy expenditure can cause growth retardation to reach adult stage and reproduce by oviparous or ovoviviparous way. It is important to consider a carbohydrates supply in diet by adding rice or wheat bran, as well as heterotrophic bacteria contribution of such as those produced in biofloc systems [44].

Some authors mentioned the importance of supply a carbon/nitrogen supplement in high salinities culture medium to increase growth, maturity and fecundity in *Artemia* organisms [45]. These authors used pig and tapioca compost to increase C/N ratio and observed the cysts production (9.96 kg wet weight per hectare) with respect to control experiment with only 2.84 kg (wet weight per hectare). The supply of adequate fertilizer in this crustacean culture medium to increase C/N ratio, not only increase microalgae grow but heterotrophic bacteria grows too in total water column, which can be consumed for these organisms and can obtain their optimal energy content to make their metabolism functions, under another unlike physical and chemical conditions in culture medium. Addition of C/N source in a 20/1 ratio increases cysts production per female in 24-90 range, consequently in 120 m^2 pond can be achieved a 28-38 kg cysts wet weight $month^{-1}$ production; this amount is similar to 23-35 kg cysts wet weight $month^{-1}$ obtained by extrapolating this experiment data to 120 m^2 pond [46].

An important variable was the amount of inoculated nauplii in natural habitat or ponds to obtain a culture success [47]. Supply of 3 to 5 x 106 nauplii m^2 day^{-1} allow a biomass production of 5 kg 1000 m^2 day^{-1} and a cyst production of 2 kg 1000 m^2 $month^{-1}$. Extrapolating information, laboratory conditions culture system of this study give an amount of 6.5-9.8 kg 1000 m^2 day^{-1} biomass and 2.9-4.3 kg $month^{-1}$ cysts. Other authors mentioned that cyst production in *Artemia* culture system depends on various factors such as broods number, salt concentration, temperature and oxygen availability in medium, photoperiod, and even Fe content from food [9]. These authors mentioned too that lower cysts production is caused by the stability in culture medium in their physical and chemical conditions that maintain ovoviparity stage in these populations. This condition may be a selective advantage to intraspecific competition, because this encystment mechanism of embryos cause a grow retarding in *Artemia* sp. populations. The obtained results in this experiment of reproductive potential (biomass and cysts production per female), cannot

match with the potential estimation in specific habitat or culture condition, because the production must be influenced for multiple factors and each one of *Artemia* sp. population respond different at each variable, like salinity concentration, oxygen percentage saturation, high population densities per culture litter and lowest concentration of food at culture medium [9]. These authors mentioned that food concentration in culture medium is essential to encyst embryos. They found a cysts production of 34 cysts per female at 80 gL^{-1} salinity culture test and 15-127 cysts range production of cysts per female in 120 gL^{-1} salinity. Different values were obtained in this experiment, because it was not found cyst production at 80 gL-1 salinity test and in 120 gL^{-1} salinity of culture medium it were obtained 57 to 66 cysts per female. Some authors explain that the most important factor to induced *Artemia* sp. to produce cysts was the photoperiod [48]. The experiments who have more darkness period and temperature up to 25 °C, produce more cysts per female. At temperatures lower than 25 °C the principally type of reproduction is the ovoviparity, being the same in culture medium with continuous light. The experiment with these Mexican *Artemia* population which did not had this darkness period, allowed that at high salinities (>120 gL^{-1}) in culture medium, the ovoviparity type of reproduction were constant. These authors mentioned that availability of food and oxygen concentration are not critical variables to change oviparity reproduction in *Artemia* females, but are important for growth and amount of reproductive structures (nauplii or cysts) [48].

Birth number (nauplii or cysts) were correlated with light intensity in culture mediums, because type of reproduction can be modified not only by the type of light but intensity too [49]. These authors confirm that oviparity increase when light intensity is lower in culture medium (57.92% in 0 lux; 22.65% at 5,000 lux). They work with *A. urmiana* specie and observed that number of births have a production of 685 cysts and 935 nauplii above 100 lux, meanwhile under that light intensity it decreased to 217 cysts and 234 nauplii. The cysts production increase when the *Artemia* females are placed to 2,000-5,000 lux source, due to organism present active swimming and their gregarious behavior induced them to reproduction.

Some authors mentioned that culture beaker or pond depth was a variable which induced *Artemia* populations to produce cysts [50]. These authors point out that the cysts production decreases when water level reaches 40 cm deep or more; in the same way, they note that cysts production begins when 95 gL^{-1} salinity medium reaches [51]. In this experiment it was found that below 100 gL^{-1} salinity cysts not occurred.

Biomass production of 15.72 gL^{-1} in 15 culture days can be obtained, after they inoculated 10 nauplii mL^{-1} in 1.5 L beakers [52]. In this experiment, lowest obtained values were 154-411 g $160L^{-1}$. Other authors, in 700 L culture medium beakers obtained 26.45 to 33.86 g of cysts and 813.6 to 1,226.7 g of biomass in 38 culture days [53]. The values were very similar to ones obtained in this experiment with 13.12 to 30.62 g of cysts and 673.75 to 1,798.12 g of biomass (considering 700 L culture beakers), but only in 21 culture days. *A. franciscana* cultured at 100 gL^{-1} salinity obtained 162 cysts per 40 females, at 120 gL^{-1} salinity culture medium produced 196 cysts per 40 females and in 140 gL^{-1} salinity test, there are not cysts production because *Artemia* population die [54], unlike to this experiment that obtained 2,080 to 2,600 cysts per 40 females in 100 gL^{-1} salinity test, 2,200 to 2,640 cysts per 40 females at 120 gL^{-1} and 2,680 to 3,400 cysts per 40 females at 140 gL^{-1}.

Finally, the biological and economic implications that values of cysts and nauplii production obtained in this experiment allow better managements in natural habitat of Mexican *A. franciscana* populations to maintain the biodiversity and ecology conservation of this specie, but also a commercial exploitation in laboratory or natural biotopes to supply the local Mexican country aquaculture or aquariophylia industry.

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