academicJournals

Vol. 7(7), pp. 210-217, July, 2015
DOI: 10.5897/JENE2015. 0529
Article Number: 18C4DCF54377
ISSN 2006-9847
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http://www.academicjournals.org/JENE

Journal of Ecology and the Natural Environment

Full Length Research Paper

Salinity effects on growth of four *Artemia franciscana* (Kellogg, 1906) populations, cultured in laboratory conditions from Yucatan Peninsula

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Received 2 June, 2015; Accepted 19 June, 2015

The aim of is study was to determine the growth performance of Mexican *A. franciscana* Yucatan Peninsula strains in different salinity tests. Four populations from different habitats were studied: Real de las Salinas (RSAL), Cancun (CAN), San Crisanto (CRIS) and Celestun (CEL). Nauplii from each population were inoculated in 200 L plastic tanks with 160 L of dissolved rock salt water at 40, 60, 80, 100 and 120 g L^{-1}. The organisms were fed with *Tetraselmis* sp. and *Pinnularia* sp. microalgae (500 x 10^3 cells mL^{-1} water concentration) during the experiment period. Biometry length was measured to obtain absolute growth rate (AGR), instantaneous growth rate (IGR), and length gain (LG) values. Variance test (ANOVA) analysis was applied to determine the significant differences (P <0.05) between final length and growth rates of four populations. The total length range was 6.675-9.589 mm; AGR values ranged between 0.395 and 0.571 mm per day. IGR percentage range was 17.623-18.91% increase per day, and length range gain values were 6.277 to 9.130 mm. No significant difference was shown between 100 g L^{-1} salinity test and 120 gL^{-1} test, and then between CEL and RSAL strains. The salinity variable showed high percentage (62 to 93%) in the salinity tests or significant strains (p<0.05). Yucatan Peninsula has several salt ponds with natural *Artemia franciscana* sources that can be used for aquaculture or aquarium industry. It is important to know growth performance of species in specific salinity concentration. This will help one to understand the adaptation of *Artemia* populations cultured in laboratory and also ensure better culture system management for producing biomass for use in aquaculture or aquarium industry or academic laboratory centers.

Key words: *Artemia franciscana,* survival, growth rates, length gain, salinity.

INTRODUCTION

Many aquatic organisms and crustacean, in particular, have wide salinity tolerance range in their environment. As a result, it is necessary to have an ion balance between the flow of ions from external environment to their internal hemolymph through osmoregulatory mechanisms. This ensures optimal ion regulation and therefore better growth rates (Abatzopoulous et al., 2002a). One of these organisms that can survive different salinity concentrations is crustacean branchiopod *Artemia*. It has two main structures responsible for carrying out the

process of osmoregulation in adults: the gut epithelium (Croghan, 1958b); Plattner, 1955) and meta-epipodites in branchial segments (Copeland, 1966; Croghan, 1958a). Croghan (1958a) suggested that the inlet continuous liquid medium toward intestine gut can also take in sodium chloride (NaCl) and water, both necessary for organisms' survival.

Artemia is among the few organisms adapted to survive in very diverse living conditions. It can survive in salinities as low as 10 gL^{-1} (Abatzopoulous et al., 2006 a,b) and as high as 340 gL^{-1} (Post and Youssef, 1977). This is a wide range of salinity concentrations in natural habitat that can be used under laboratory conditions. That is why it is necessary to know the narrow salinity range in order to obtain better growth and survival results. It will also help in biomass or cysts productions from different *Artemia* populations that respond adequately to the stress of ions dissolved in medium, and osmotic pressure of internal fluids (Copeland, 1967).

Castro et al. (2013) mentioned that in Mexico, coastline and inland waters have many natural salt water bodies, both in Gulf of Mexico and Pacific Zone. Unfortunately, many of them do not produce salt anymore and *A. franciscana* populations have disappeared from these habitats. Another problem is the low budget on field investigations for knowing the presence or absence of *Artemia* populations in all these salt bodies in Mexico. In few natural habitats, the local salt producers collect *Artemia* biomass by hand and sell to aquarist industry or local aquaculture farmers (Castro et al., 2013).

In the year 2000, in Mexico, three *Artemia* inland populations were described: they originated from Coahuila, San Luis Potosí States, and Texcoco township; nine populations localized in Pacific, Gulf of Mexico and Yucatan Peninsula coastal waters. Torrentera and Dodson (1995) presented the biometry values of four populations of *Artemia* localized in Yucatan Peninsula manmade saltworks. The cysts were collected during 1990-1992. Torrentera and Abreu-Grobois (2002) studied these same populations from cytogenetic variability and differentiation angle. Torrentera and Dodson (2004) highlighted weather conditions of the habitat, phyto-plankton, bacteria and birds (flamingos), populations' dominance, also cyst and biomass productions from these *Artemia* Yucatan sites.

Maldonado-Montiel and Rodriguez-Canche (2004) studied the biomass production of *A. franciscana* population located in "Real de las Salinas", Campeche State, but they did not mention the geographical location of this habitat. Rodriguez-Canche et al. (2006) mentioned the geographical localization of "Real de las Salinas", Campeche. The cysts were collected from 1997-2000. The authors described some biological characteristics and biochemical composition of cysts and nauplii.

Castro et al. (2010) studied five Pacific Coast Mexican populations and mentioned the geographical localization of these *Artemia* strains. Castro et al. (2010) described recently the last habitat of *Artemia* population found in Mexico; their geographical localization is a little saltwork near Cancun, Quintana Roo. The authors studied the reproductive potential of two populations from Pacific coast: two populations from Yucatan Peninsula and two populations from Inland waters in a culture medium of 100 and 120 g L^{-1} salinity.

Therefore, it is important to determine salinity response of each Mexican *Artemia* strain to ascertain optimum conditions for their growth and understand their adaptation pattern to varying salinity.

The aim of this study was to know how length rates and length gain variables correspond with specific salinity concentrations cultured under laboratory condition. The results obtained from the study will be useful for the potential use of these strains in manmade saltworks operations as well as aquaculture and aquarium industry.

MATERIALS AND METHODS

Strains used in the experiment

This study was performed in Live Food Production Laboratory at Autonomous Metropolitan-Xochimilco University, Mexico. The dehydrated and cold (5°C) storage cysts (0.5 g) of four *Artemia franciscana* strains (Table 1, Figure 1) from Yucatan Peninsula Coastal Zone were hatched under 40 g L^{-1} of salinity, pH of 8-10 and 25±2°C temperature, with constant illumination and air supply (Castro et al., 2003).

Culture experiments

The nauplii hatched were siphoned into separated beakers and then transferred to 200 L plastic tanks with 160 L of different salt concentrations (40, 60, 80, 100 and 120 g L^{-1}). They were measured with a Vee Gee STX-3 refractometer; pH of 8-10 was tested with a HANNA pH meter. They were kept under constant illumination (white light tube, 40 watts), air supply (>2 mg O_2 L^{-1}) and temperature (25±2°C). *Artemia* nauplii density was adjusted to one individual per 1 mL to avoid growth problems for space. The animals were fed *ad libitum* with microalgae *Tetraselmis* sp. and *Pinnularia* sp. at 500 x 10^3 cells mL^{-1} culture density (1 L each) during their pre-adult stage (between 14-21 days).

Total length biometry

In the salinity culture where the organisms reached adulthood (when first mating was observed), fifty females and fifty males from each population were obtained and maintained separately for two weeks in 4 L beakers. The beakers have the same salinity, 250 mL

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Table 1. List of Yucatan peninsula Mexican *Artemia* strains studied, abbreviation used and geographical location.

Site	State	Abbreviation	Co-ordinates
Cancun	Quinatana Roo	CAN	21°10’ N; 86°47’ W
San Crisanto	Yucatan	CRIS	21º 21’ N; 89º 07’W
Celestun	Yucatan	CEL	20º 52’ N; 90º 23’W
Real de las Salinas	Campeche	RSAL	20°02’ N; 90°14’ W

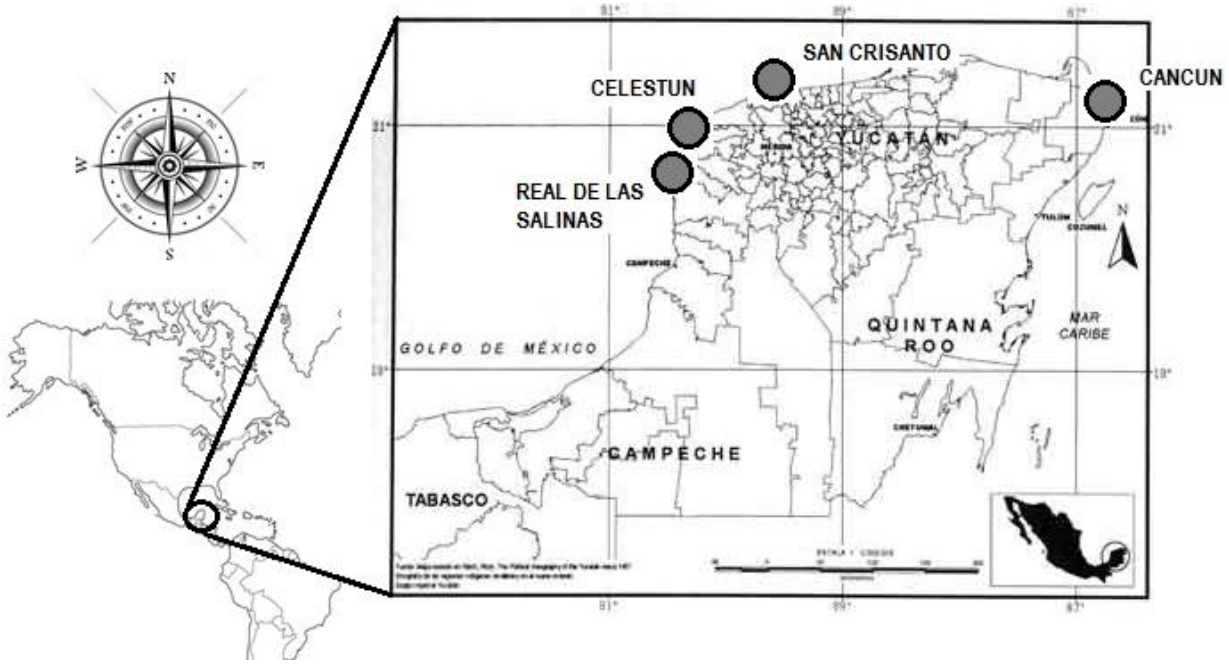

Figure 1. Geographical location of Yucatan peninsula coast, Mexico strains habitat.

of microalgae´s culture medium (125 mL from each microalga), pH of 8-10 and constant illumination and air supply. This is to allow individual growth without expending energy on reproduction. After two weeks, the adults were fixed with few drops of acetic acid. The total length biometry was measured with an optical microscope DM 750 Koheler Leica equipped with a color camera ICC50 HD Leica having Interactive Measure Module.

Growth rate analysis

The Absolute Growth Rate (AGR) formula used was:

$$AGR = \frac{\text{Final length - Initial length}}{\text{Total experimental days}}$$

Wooton (1991)

The Instantaneous Growth Rate (IGR) formula used was:

$$IGR = \frac{\text{NL Final length - NL initial length}}{\text{Total experimental days}} \times 100$$

Soriano and Hernandez (2002)

NL = natural logarithm.

For length gain (LG), the formula used was:

$$LG = \text{Final length - Initial length}$$

Moreno et al. (2000).

Statistical analysis

A Microsoft and Box Plot techniques were performed to ensure that the assumption of normality was held for each parameter using Excel 2010 (Microsoft Corp., Washington, USA). Mean values and standard deviation for total length biometry were made with descriptive statistical analysis. One way ANOVA was used to determine if there was a significant difference (Kachigan, 1991) between the strains. The pairwise comparison Tukey method (p=0.05) was used to compare pairs of sample means. Type classification was based on population grouped according to their specific salinity cultured medium (Sokal and Rohlf, 1981; Kachigan, 1991). The SYSTAT 13 (Systat Software Inc., California, USA) software package was used for statistical analysis.

Table 2. Mean values and ± SD of total length (in mm) of Mexican Yucatan peninsula *Artemia* strains studied.

Strain	Culture salinity test			
	60 g L^{-1}	80 g L^{-1}	100 g L^{-1}	120 g L^{-1}
Cancun	7.344 ± 0.249	6.675 ± 0.048	8.093 ± 0.088[1]	8.205 ± 0.206[1]
San Crisanto	8.161 ± 0.324	7.496 ± 0.132	9.042 ± 0.098[1]	9.139 ± 0.225[1]
Celestun	8.577 ± 0.187[a]	7.756 ± 0.145[a]	9.368 ± 0.081	9.531 ± 0.136[a]
Real de las Salinas	8.646 ± 0.342[a]	7.816 ± 0.147[a]	9.432 ± 0.063	9.595 ± 0.138[a]

Same letter in column did not show significant differences (p>0.05). Same number in a row did not show significant differences (p>0.05).

Table 3. Mean AGR values and ± SD of millimeters per day of Mexican Yucatan peninsula *Artemia* strains studied.

Strain	Culture salinity test			
	60 g L^{-1}	80 g L^{-1}	100 g L^{-1}	120 g L^{-1}
Cancun	0.434 ± 0.016	0.395 ± 0.018	0.480 ± 0.006[1]	0.489 ± 0.013[1]
San Crisanto	0.482 ± 0.020	0.444 ± 0.020	0.537 ± 0.006[1]	0.543 ± 0.014[1]
Celestun	0.507 ± 0.012[a]	0.460 ± 0.021[a]	0.557 ± 0.006	0.567 ± 0.009[a]
Real de las Salinas	0.511 ± 0.021[a]	0.463 ± 0.020[a]	0.561 ± 0.005	0.571 ± 0.009[a]

Same letter in column did not show significant differences (p>0.05). Same number in a row did not show significant differences (p>0.05).

RESULTS

At 40 g L^{-1} salinity test, the final length biometry values cannot be taken because all individuals from each population died at juvenile stage.

Total length biometry

The total length means ±SD (in mm) of Yucatan Peninsula *Artemia* strains are shown in Table 2.

In all the populations, the highest values were observed at 120 g L^{-1} salinity test and the lowest length values at 80 g L^{-1} salinity test. The ANOVA test showed no significant differences between CEL/RSAL populations at 120 g L^{-1} (p=0.521), 80 gL^{-1} (p=0.251) and 60 g L^{-1} salinity experiment tests (p=0.779). In 100 gL^{-1} salinity test, all populations showed significant differences between them (p<0.001). With respect to significant differences between salinity tests at same population, only CAN (p=0.056) and CRIS (p=0.296) populations did not show significant differences at 100/120 g L^{-1} salinity test. The variation percentage was 35% for the population; 60% for the salinity and only 0.23% for the interaction between these two variables.

Absolute growth rate (AGR)

The AGR values are shown in Table 3. The highest values were at 120 g L^{-1} salinity and the lowest values at 80 g L^{-1} salinity culture test. The highest values were in RSAL strain with 0.571 mm per day and the lowest one was shown in CAN strain with 0.395 mm per day. The ANOVA test showed significant differences between salinity tests at same strain; only CAN and CRIS strains did not show significant differences at 100/120 gL^{-1} salinity tests (p=0.056 and p= 0.296 respectively), also between CEL/RSAL strains at 60, 80 and 120 g L^{-1} salinity tests. The variation in significance was 62.58% for salinity, 32.71% for the strain and only 0.24% for the interaction between salinity and strain variables.

Instantaneous growth rate (IGR)

The final percentage rates are shown in Table 4. The highest values were at 120 g L^{-1} salinity and the lowest values at 80 g L^{-1} salinity. The highest values were recorded for RSAL strain with a length increase of 18.918% per day and the lowest value was for CAN strain with 17.623%. ANOVA test did not show significant differences between salinity tests and strains variables (p>0.05). The variation of significance is given by the salinity with 93.19%.

Length gain

The final length gain values are reported in Table 5. The

Table 4. Mean IGR values and ± SD in percentage of Mexican Yucatan peninsula *Artemia* strains studied.

Strain	Culture salinity test			
	60 g L^{-1}	80 g L^{-1}	100 g L^{-1}	120 g L^{-1}
Cancun	18.217 ± 0.213	17.623 ± 0.045	18.827 ± 0.068[1]	18.911 ± 0.158[1]
San Crisanto	18.163 ± 0.248	17.635 ± 0.110	18.808 ± 0.068[1]	18.873 ± 0.153[1]
Celestun	18.270 ± 0.137	17.642 ± 0.116	18.822 ± 0.054	18.930 ± 0.089
Real de las Salinas	18.263 ± 0.248	17.636 ± 0.117	18.811 ± 0.042	18.918 ± 0.090

Same number in a row did not show significant differences (p>0.05).

Table 5. Mean length gain values and ± SD (in mm) of Mexican Yucatan peninsula *Artemia* strains studied.

Strain	Culture salinity test			
	60 g L^{-1}	80 g L^{-1}	100 g L^{-1}	120 g L^{-1}
Cancun	6.946 ± 0.249	6.277 ± 0.048	7.695 ± 0.088[1]	7.807 ± 0.206[1]
San Crisanto	7.715 ± 0.324	7.050 ± 0.132	8.596 ± 0.098[1]	8.693 ± 0.225[1]
Celestun	8.116 ± 0.187[a]	7.295 ± 0.145[a]	8.907 ± 0.081	9.070 ± 0.136[a]
Real de las Salinas	8.181 ± 0.342[a]	7.351 ± 0.147[a]	8.967 ± 0.063	9.130 ± 0.138[a]

Same letter in column did not show significant differences (p>0.05). Same number in a row did not show significant differences (p>0.05).

highest values were at 120 g L^{-1} salinity culture test with a range of 7.807 to 9.130 mm and the lowest values at 80 g L^{-1} salinity culture test, with 6.277 to 7.351 mm range. The highest values were observed for RSAL strain with a length gain of 230.74% and the lowest one for CAN strain with 195.71%. The variation of significance of ANOVA test is given by salinity variable with 62.58%, strain variable with 32.17% and interaction between these two variables with 0.24%.

DISCUSSION

In this study, *Artemia* strains from Yucatan Peninsula exposed in culture medium with salinities below 60 g L^{-1} died in metanuapliar stage, because the osmoregulatory mechanisms were correctly functional at 60-120 g L^{-1} salinity range. The length differences between Mexican strains are not due to their habitat origin, but are triggered by salinity variable. Sayg (2004), who worked with parthenogenetic *Artemia* populations, suggested that these differences can be considered as local biotope response and not only salinity intrapopulation response. This was seen in their study in the ploidy level strain and perhaps in larval energy content, in our study.

Other authors such as Chapman (1968); Metalli and Ballardin (1972); Vanhaecke and Sorgeloos (1989) indicate that genetic variability may induce strain damping with respect to extreme conditions such as salinity. Vanhaecke et al. (1984) also found low survival at 35 g L^{-1} and increased survival in salinities above 90 g L^{-1}. Post and Youssef (1977) indicate that *Artemia* culture at salinities <45 g L^{-1} decreases survival; Hammer and Hurlbert (1992) observed that juveniles of different *Artemia* species and strains grow slowly and adults die at < 38 g L^{-1} salinity. However, El- Bermawi et al. (2004) found that Egyptian *Artemia* has a survival of 60% at salinity of 35 g L^{-1}. Comparing data with other species of *Artemia* genus like *A. salina*, *A. sinica*, *A. persimilis* and some parthenogenetic populations of *Artemia* (Browne and Wanigasekera (2000) a 24% survival was observed at 60 g L^{-1} salinity. Van Stappen et al. (2003) found only a 39% survival of *A. tibetiana* at 35 g L^{-1}. Sayg (2004) found a 15% survival of *A. parthenogenetica* at salinities below 80 g L^{-1} from Turkey and Greece; Agh et al. (2008) and Abatzopoulos et al. (2006a,b) did not obtain survival results with *A. urmiana* at 50 g L^{-1} salinity tests. With regard to American Continent *Artemia* species, Medina et al. (2007) found *A. persimilis* survival rates of 5.3% only at 30 g L^{-1} of salinity.

The consulted literatures showed that the salinity range of 80-120 g L^{-1} was considered as the most appropriate to inoculate the hatched nauplii, except with the species *A. tibetiana*. In inoculating the Mexican nauplii at salinities above 120 g L^{-1} culture, Yucatan Peninsula's populations show 100% mortality because at this stage they cannot activate the proper enzymes to avoid osmoregulatory mechanism (Clegg and Trotman, 2002). Dana and Lenz (1986) showed that survival decreases below 20% at salinity above 179 g L^{-1}; and the osmoregulatory apparatus is damaged not only by ion concentration, but also by frequency and time duration of salinity concentration

in their own habitat. *A. salina* cultivated in a range of 150-200 g L^{-1} salinity showed 100% mortality; *A. urmiana* showed 100% mortality when it was inoculated at 200 g L^{-1} salinity (Agh et al., 2008; Abatzopoulos et al., 2006b).

Salinity concentrations < 60 g L^{-1} and > 120 g L^{-1} affect the survival of Mexican *Artemia* Yucatan Peninsula's populations; the same result has also been observed for other *Artemia* species: *A. urmiana*, *A. persimilis*, and *A. tibetiana.* It is possible to maintain *Artemia* cultures in a laboratory if salinity increases gradually (10 g L^{-1} every week) to allow the establishment of the enzymatic activity in osmoregulatory mechanism in the culture organisms (Post and Youssef, 1977; Wear et al., 1986; Triantaphyllidis et al., 1995; Van Stappen, 2002; Agh et al., 2008) like in natural habitat. Tackaert and Sorgeloos (1991) mentioned that genetically imprinted factors exist that respond to salinity changes in each *Artemia* species or strain; and they function better at 100-180 g L^{-1} salinity. This can be seen in Mexican Yucatan Peninsula strains, showing 80% of significance variability to salinity tests. This information allows one to make better laboratory culture management of this crustacean where the salinity, temperature and food variables can be controlled (Wear and Haslett, 1987).

In studying the growth of *A. franciscana* Mexican Peninsula's strains at different salinities, there were observed significant differences between salinity tests, the strains and the interaction between these two variables. The same applies with AGR and IGR final data, where the percentage variability with respect to the salinity was between 81-88%; the strain variable was 9-17% and the interaction between these two variables was 1.40-1.89%. The differences between strains are mainly due to the size of the nauplii. This biometry variable was genetically determined (Sorgeloos et al*.,* 1976; Vanhaecke and Sorgeloos, 1980); and therefore this daily growth (in millimeters or percentage) has similar proportion between salinity tests, but the final length was different.

Mexican Peninsula's *A. franciscana* strains increase their total length with <80 g L^{-1} salinity tests. Only few salinity studies were done with *A. franciscana* or other species and their effect on growth increase or decrease and AGR and IGR rates. Amat et al. (2004) found that *A. persimilis* (Argentina) showed a total length range of 9.3-10.2 mm at 70-80 g L^{-1} salinity culture test. El- Bermawi et al. (2004) reported 5.79 mm at 120 g L^{-1} culture salinity and 5.41 mm at 80 g L^{-1} salinity concentration. Rodríguez-Almaraz et al. (2006) said *A. franciscana* from Baja California, Mexico hadn 6.27 to 7.88 mm length range at 115-195 g L^{-1} salinity medium. Medina et al*.* (2007) mentioned 8.8-10.0 mm length range in populations cultured at 120 g L^{-1} salinity. Dana and Lenz (1986) found *A. monica* specie to have 6-8 mm length range at 76-118 g L^{-1} salinities. For *A. urmiana*, Arashkevich et al. (2009) reported a total length of 6-13 mm in a range of 100-110 g L^{-1} salinity. Sayg (2004) reported 8.31-10.92 mm for parthenogenetic population(s) of *Artemia* in 160 g L^{-1} salinity culture test. It is noteworthy that organisms of parthenogenetic population(s) of *Artemia* always are larger than bisexual species.

Vanhaecke et al. (1984) and Sayg, (2004) mentioned that smallest length at 80 g L^{-1} culture salinity (7.93 mm) is caused by malfunction of enzymes and energy levels involved in osmoregulatory mechanism. This increased at 100 g L^{-1} salinity to 8.70 mm, and 9.72 mm at 120 g L^{-1} salinity in Mexican Peninsula *A. franciscana* strains. Above 140 g L^{-1} salinity culture test, Gilchrist (1960); Baid (1963); Triantaphyllidis et al. (1995) and El- Bermawi et al. (2004) indicated that the effect is inversely proportional when the salinity increases. This statement could not be observed in the Mexican Peninsula strains because the nauplii died in these salinity (140, 160 and 200 g L^{-1}) culture test.

With respect to growth rates (AGR and IGR), Sayg (2004) mentioned that length and growth rate are not affected when salinity increase occurs gradually in culture recipes. Triantaphyllidis et al. (1995) indicated that differences in length and growth rates are significant in parthenogenetic populations of Tanggu (China). Abatzopoulos et al. (2006a,b) observed in *A. urmiana* specie that growth rate is not affected with increased salinity.

Although Agh et al. (2008) disagreed with that because they found best growth rate at 75-100 g L^{-1} and those values changed when salinity increased. Mayer (2002), who studied *A. franciscana* populations from Dominican Republic and Puerto Rico, found that length differences are mainly due to geographic isolation and their particularly ecological adaptation to their habitat.

Cole and Browne (1967) and Hontoria and Amat (1992) mentioned that the ionic composition of water affects *Artemia* morphology and length; Vanhaecke and Sorgeloos (1980) mentioned that *Artemia* length responded to its environmental conditions and those changes were considered as local adaptation to its own habitats. In recent works, Asem and Rastegar-Pouyani (2008) mentioned that to find differences in length and growth rates it is better to use the male organisms for *Artemia* populations, which respond with greater variation to salinity increase. Bowen et al. (1985) and Castro (2004) mentioned that due to length differences between *Artemia* strains at different salinities, a long-range time process may provoke speciation process. This might be due to lack of successful interbreeding between strains (caused by length differences between male and females organism), leading to the onset of incipient species or related species

From the above, it is not advisable to generalize the local *Artemia* species or strain obtained with respect to salinity and growth rates whether they are the same species (different strains) or much less different species (Litvinenko et al. (2007); Agh et al*.* (2008) and Naceur et

al. (2009). The knowledge of the best salinity tests that favour the Mexican strains' growth rates under laboratory conditions allows one to manage well natural habitats applications to avoid salt, food and culture time waste. It helps one to get bigger and healthy *Artemia* organisms (biomass), to sell to the aquaculture or aquarist industry near their natural habitats. It can also provide income to those who work in those sites, making it possible for them to own their production systems. It will lead to better management of academic laboratories which make experiments with those populations.

Conclusion

Mexican *A. franciscana* from Yucatan Peninsula habitats died when they were cultivated in 60 g L^{-1} salinity under laboratory conditions. Growth performances increase when salinity increases in culture medium. Better performance is observed at 100 and 120 g L^{-1} salinity concentrations. This information allows one to know better culture management of these *Artemia* populations either in natural habitats or laboratories of academic centers.

Conflict of interests

The authors did not declare any conflict of interest.

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