Effect of Temperature and Salinity on Survival and Growth of *Artemia franciscana* from Basrah, Iraq.

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Abstract

Laboratory experiments were carried out to express the effects of temperature, salinity and the combined effects of temperature–salinity on survival, growth and biomass of *Artemia franciscana* at 5 temperatures: 10, 20, 25, 30 and 35 °C and 5 salinities: 35, 55, 75, 95 and 115 ‰. Temperature of 20 °C and salinity of 55 ‰ showed best survivals (93 %), biomass (122.87 mg DW/L) and high growth (4.94 mm), whereas at 30 °C and salinity of 95 ‰, the least survivals (21 %) was attained. However, the highest average growth was 6.1 mm attained at 30 °C and 115 ‰, and the lowest growth was 1.49 mm at 10 °C and 95 ‰, meanwhile, the lowest average biomass was 3.79 mg DW/L at 10 °C and 115 ‰. Apparently, temperature of 20 °C and salinity of 55 ‰ is the best combination for the local strain of *A. franciscana* from Basrah and is recommended for mass culture of this strain.

Key words: Survival, growth, biomass, *Artemia franciscana,* temperature, salinity

Introduction

The brine shrimp *Artemia* was long been considered as the most adapted invertebrate, as it's strains were found in very widely different geographic habitats of the five continents in the tropical, subtropical as well as temperate regions (Persoone and Sorgeloos, 1980), which were adapted to live in greatly fluctuating temperature and salinity (Bowen *et al.*, 1978). These adaptations made the brine shrimp dominating the most extreme habitats; it has inhabited highly saline lakes, temporary pools subjected to wide variations in the ecological conditions (Engel and Angelovic, 1968) and Saharan (Ben Naucer *et al.*, 2009). It is expected, therefore, that these changing habitats in temperature and salinity would lead to variation among these populations at their limits of tolerance (Vanhaecke *et al.*, 1984). Abreu-Grobois and Beardmore (1982) pointed out to the genetic variation reported in the *Artemia* strains which may lead to the origin of physiological (functional) differences in the thermal and salinity tolerance among the different strains. Moreover, the optimum effect of salinity- temperature interaction may be different from strain to the other (Sorgeloos et al., 1976) and this may, probably, be due to the presence of genetically controlled traits (Vanhaecke *et al.*, 1980). However, Vanhaecke *et al.*(1984) emphasized that there were strong and clear evidences indicating that these differences in the thermal limits of tolerance are linked to genetic variation among and within the strains or populations of the species.

Furthermore, Irwin *et al.*(2007) suggested that, in spite of the tolerance of *Artemia* to broad limits of temperature and salinity, yet both temperature and salinity are major determinants of survival, growth and reproduction which may lead to the evolution of different phenotypes according to location. But Van Stappen (1996) who emphized that each of temperature and salinity had important effect on survival and growth of all strains of *Artemia*, stressed that the effect of temperature is more pronounced. Williams and Geddes (1991) clearly indicate that temperature and salinity are among the most important physical factors in the biology of marine and brackish water organisms, and there is a complex relation between these two factors, as temperature may influence salinity effect and changing the limits of tolerance of the organism to salinity and vise versa.

Helfrich (1973) considered *Artemia* as a crustacean with wide range of temperature tolerance (Eurythermal) and salinity (Euryhaline), but most of the geographical strains (races) did not show the ability to withstand temperature less than 6 °C, whereas the highest temperature tolerated is nearly 35 °C. For salinity, however, most populations can withstand salinity concentrations between 33-40 ‰ (Baid, 1965). Coincided with this, Post and Youssef (1977) found that *Artemia* are unable to survive long time exposure to salt concentrations of 340 ‰, whereas, the lower limits of salinity favored by *Artemia* to live in are mostly in habitats ensuring to avoid predation (Persoone and Sorgeloos, 1980). Although *Artemia* had the capability to live in water of salinity ranging from 5-35 ‰, yet the absence of any means of defense against predation made them easy to be preyed upon by animals living in the same range of salinity. Hence, in their physiological adaptation *Artemia* tend to live in environments with salinity levels above the upper limits of tolerance of the predators (Van Stappen, 2002).

Several studies were carried out to investigate the importance of these two factors on different strains of *Artemia* in order to define the limits of temperature or salinity or both affecting the biology of *Artemia* or their various larval stages. At temperature of 20 °C and salinity of 70 ‰ *Artemia* *franciscana*, had achieved good growth, doubled of that achieved at Lake Crass Mier, at the same temperature and salinity of 32 ‰ (Von Hentig, 1971). Moreover, Baid (1963) reported higher growth rates of females *Artemia* maintained at salinity of 12.5 ‰ than those at 65 ‰, whereas Sick (1976) obtained a growth of 6mm by *Artemia* during 16 days at 25 °C and 33 ‰ and Royan (1980) observed maximum length attained by an Indian strain of parthenogenetic *Artemia* during 19 days at 27 °C and 140‰.

Laboratory observations on several species of *Artemia* indicate that the optimum limit of salinity tolerance was from 60-150 ‰ (Baxevanis *et al*., 2004; Browne and Bowen, 1991; Triantaphyllidies *et al*., 1995,). However, in their investigation on the response of 13 different strains of *Artemia* to different salinity concentrations, Vanhaecke *et al*. (1984) observed great mortalities among most of the strains at 5 ‰ except larvae of an Argentinean strain which did not affected by this concentration nor by that of 15 ‰. On the contrary *A. urmiana* did not exhibit any effect on the biological traits at the rise of salinity from 100-180 ‰, whereas the increase in salinity of 120 – 140 ‰ influenced many of the species or strains of *Artemia* (Abatzopoulos *et al*., 2006; El- Bermawi *et al*., 2004,).

The limits of tolerance to high temperatures had been observed in a laboratory study on the larvae of *Artemia* from Shark Bay and Larnaka Island, Cyprus, by Vanhaecke *et al*. (1984), and found that mortalities started at 30 °C, and with the rise of temperature to 34 °C the mortalities were 100 %, on the contrary to this, *Artemia* from Great Salt Lake, attained the best survivals (70 %) at 34 °C.

The present investigation aimed at defining the limits of tolerance of *Artemia franciscana* from Basrah to salinity and temperature and the combined effects of these two factors on the survival, growth and biomass of the species for further enhancement of feeding of cultured fish and other aquatic animals, which was not been carried out here before.

Material and Methods

Effect of temperature and salinity on the survivals and growth of larvae of *Artemia franciscana*.

Cysts and adult *Artemia* were collected from a pool opposite the University campus of Garmat-Ali, on the far side of the high-way road. The process of hatching of cysts is described in Mohammed *et al*. (2010). Different salinity concentrations used in the present study were prepared from water of the pool inhabited by *Artemia*. The water was boiled and filtered through GF/C filter papers, diluted with distilled water. All larvae used in the various experiments were at the same age, i.e Instar II, so that the larvae could consum the yolk reserve remained, and cultured at a density of 1000 larvae /L. All experiments were done under laboratory conditions and in complete darkness, with water changed every two days. Five salinity concentrations were prepared (35, 55, 75, 95 and 115 ‰) with the aid of a refractometer, 20 ml of each was placed in a glass dish and 20 larvae were transferred to each dish. The larvae at each salt concentration were subjected to 5 temperatures (10, 20, 25, 30, 35 °C), 3 replicates of each experiment were made.

Larvae were fed on a species of the alga *Dunaliella sp.* which was very abundant in a pool close to that of *Artemia*. The food was given in a concentration of 125×10^4 cells/20 larvae /day. Density of alga was determined by a Haemocytometer count. At the end of the experiment which lasted for 14 days, the survivals were counted and length measurements were taken under a Wild dissecting microscope with a graduated gratical mounted in the eye-piece of the microscope. Larvae were fixed with Lugol's solution at 100 µl / 20 ml (Vanheacke and Sorgeloos, 1980). Total length was measured to the nearest 1µm, from the front of the head to the end of biffed telson, excluding the setae. Mortalities were recorded every two days and the survivorship was then calculated.

Estimation of biomass

The biomass was estimated for the remaining living individuals at the end of the experiment (14 days). The total length of these individuals were measured and then expressed in terms of weight according the expression obtained by Ahmed (2002) as follows:-

$$\text{Log } W = a + b \text{ Log } L$$

where W is the weight in mg and L is the length in mm and a and b are constants

$a = - 2.135$ and $b = 1.9$

Statistical SPSS test was used to determine significant difference, if occurred among the different treatments of temperatures and salinities ($p<0.05$), and Factorial analysis was used to indicate significant differences in the combined effects of salinity and temperature on growth, survival and biomass of *Artemia*.

Results

Effect of temperature, salinity on the survival, growth and biomass of *A. franciscana*.

Temperature:

The best survival (93 %) was exhibited at 20 °C and the least (21 %) at 35 °C (Table 1). Growth was maximum (4.57 mm) at 20 °C and minimum (1.66 mm) at 10 °C. The highest value of biomass (122.87 mg DW/L) was recorded at 20 °C, and the lowest (13.16 mg DW/L) was at 10 °C.

Significant differences ($p<0.05$) in survivals were obtained between most of temperatures, except at 10 and 25 °C. Growth was insignificantly different ($p>0.0.5$) between 10, 35 °C and between 25, 30 °C. Biomass at 20 and 25 °C differ significantly from each other and from the values at the rest of temperatures used (Table 1).

Table 1. Percent survivals, growth (mm) and biomass (mg DW/L) of *Artemia franciscana*, from Basrah at 5 test temperatures (n=20) and salinity of 55 ‰. Different letters indicate significant differences ($p<0.05$) between various treatments.

TEMPERATURE (°C)	SURVIVAL (%)	GROWTH (MM)	BIOMASS (MG DW/L)
10	68.30 a	1.663 a	13.161 a
20	93.30 b	4.568 b	122.87 b
25	78.30 a	3.826 c	73.447 c
30	48.30 c	3.910 c	47.244 a
35	21.60 d	4.433 b	26.877 a

Salinity

Although, no significant differences in growth were attained at different salinities (Table 2), yet survivals and biomass at 55 ‰ were the highest (93 % and 123 mg DW/L) and significantly different from the rest of values. However, at salinity 35 ‰, the growth was the highest (4.63 mm), but did not differ from that at the rest of salinities (Table 2).

Temperature - salinity effects on the survivals, growth and biomass of larvae of *Artemia franciscana*.

Figures 1 a-e, present the results of the combined effect of salinity and temperature on the growth, survivals and biomass of larvae of *A. franciscana*.

At 10 °C (Fig. 1 a), the highest average survival was 14.8 ± 1.0 individuals (73.3 %) attained at salinity of 35 ‰, and the lowest average was 4.0 ± 2 individuals (20 %) at salinity of 115 ‰. The highest average growth (1.737 ± 0.01 mm) was achieved at a salinity of 75 ‰ and the lowest average growth was 1.49 ± 0.03 mm at 95 ‰. Whereas the highest average biomass was 14.43

± 0.84 mg DW/L at 35 ‰, and the lowest average was 3.79 ± 2.14mg DW/ L, attained at 115 ‰ (Fig.2 a).

Table 2. Survivals (percentage), growth (mm) and biomass (mg DW/L) of *Artemia franciscana*, from Basrah at 5 test salinities (n=20) and 20ºC. Different letters represent significant differences (p<0.05) between various treatments.

SALINITY (‰)	SURVIVAL (%)	GROWTH (MM)	BIOMASS (MG DW/L)
35	76.60 a	4.633 a	91.434 a
55	93.30 b	4.568 a	122.87 b
75	85.00 a	4.499 a	108.48 a
95	83.30 a	4.279 a	96.683 a
115	71.60 a	4.554 a	93.588 a

Statistical analysis of the combined effect of salinity and temperature on the survivals, growth and biomass, at 10°C showed significant differences (p<0.05) at salinities of 95 and 115 ‰ from the 3 other salinity concentrations (Table 2).

At 20 °C (Fig. 2b), the highest average survivals were 18.6 ± 0.5 individuals (93.3 %) at salinity of 55 ‰, and the lowest value was 14.3 ± 3.1 individuals at 115 ‰. The highest average growth was 4.63 ± 0.08 mm, achieved at 35 ‰, whereas the lowest was 4.27 ± 0.21 mm attained at 95 ‰. The highest biomass (122.86 ± 9.0 mg/DW/L) was recorded at 55 ‰ (Fig. 2 b) and the lowest (91.43 ± 7.81 mg DW/L) was reported at 35 ‰.

It is evident, however, that at 20 °C and the range of salinities of, 35-115 ‰, there were no significant differences (p>0.0.5) in the combined salinity-temperature effect on the growth and biomass, whereas survivals were significantly different (p<0.05) between salinities of 75 and 115 ‰ (Figs. 1 b, 2b).

At 25 °C (Fig. 1 c), the maximum survivals (16.3 ±1.2 individuals, 81.66 %) was reached at 75 ‰ and the minimum (13.3 ± 1.5 individuals, 63 %) was exhibited at 95 ‰. The highest average growth (3.865 ± 0.08 mm) was reported at 75 ‰ and the lowest (3.774 ± 0.09 mm) was at salinity 35 ‰. The maximum value of biomass (78.08 ± 4.86 mg DW /L) was exhibited at 75 ‰,

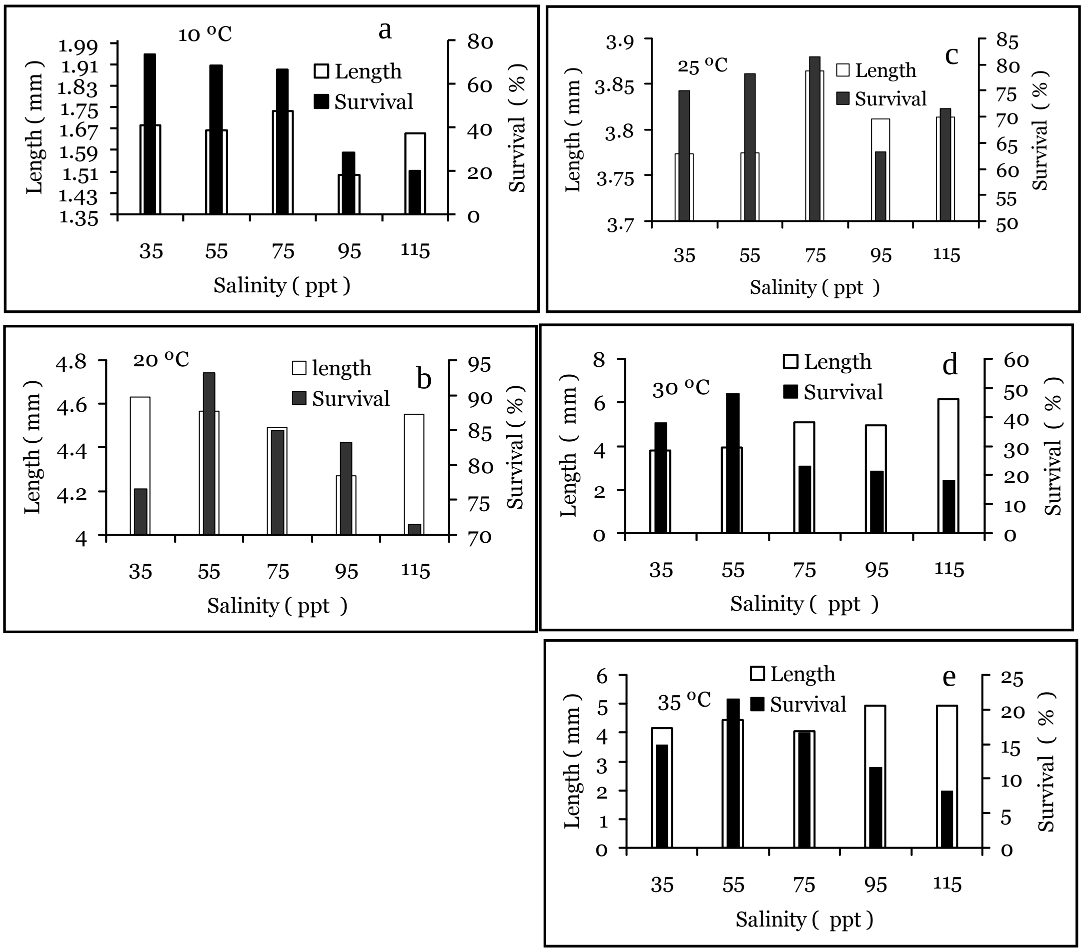

Figure 1a-e. Survival (%) and growth (mm) of larvae of *A. franciscana* at different temperatures (a at 10, b at 20, c at 25, d at 30 and e at 35 °C) and at various salinities (35, 55, 75, 95, and 115 ‰).

and the minimum (58.99 ± 4.33 mg DW/L) was attained at 95 ‰. Apparently, at 25 °C, there were no significant differences in survivals, growth and biomass at all the salinity levels tested. (Figs. 1 c, 2c).

Maximum survivals at 30 °C were 9.66±3.1 ind. (48.3 %) at 55 ‰ (Fig. 1 d), and the minimum were 3.66 ± 1.2 ind. (18.3 %) at 115‰. The highest average growth at this temperature was 6.13 ± 0.79 mm at 115 ‰ and the lowest (3.8 ± 0.13 mm) was at 35 ‰. The biomass was 47.24 ± 11.8 mg DW/L at 55 ‰ and 22.54 ± 7.20 mg DW/L at 75 ‰ (Fig. 2 d).

At 30 °C significant differences ($p<0.05$) in growth were obtained between the salinity concentrations 95, 115 ‰ and the rest of concentrations. Survivals showed significant differences ($p<0.05$) between 55 ‰ and 75, 95 and 115 ‰,

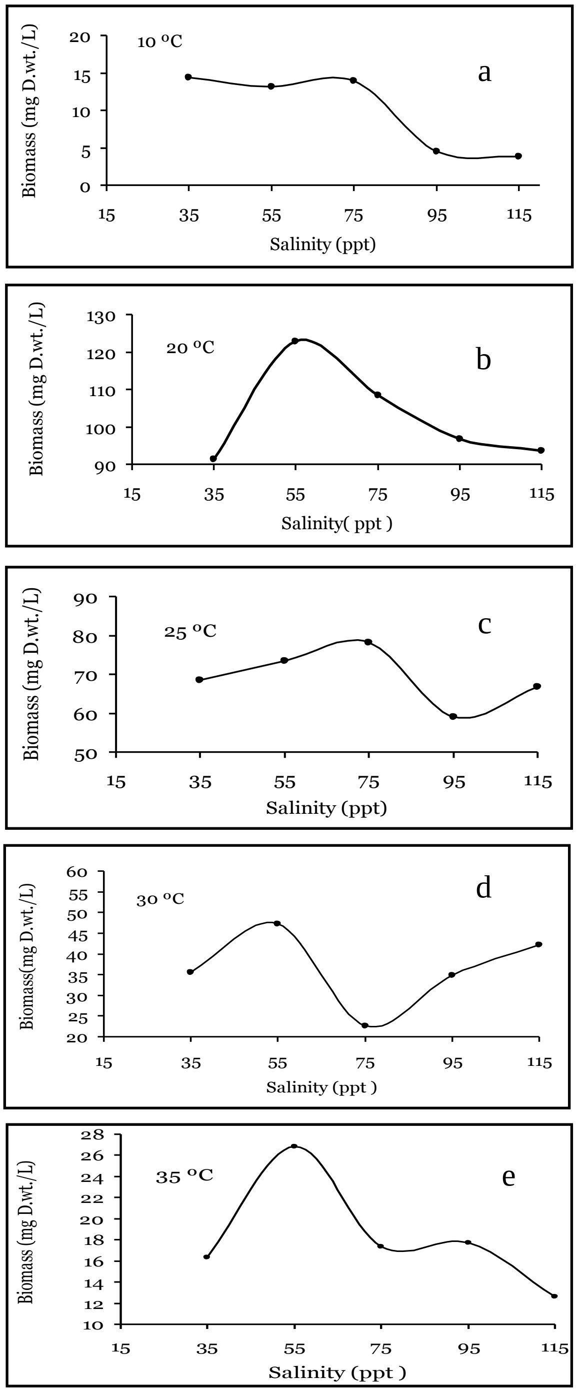

Figure 2. Biomass (mg DW/ L) of larvae of *Artemia franciscana* at 5 different temperatures (10, 20, 25, 30, and 35 °C and 5 salinities (35, 55, 75, 95, and 115 ‰).

whereas, the biomass exhibited significant difference between 55 and 75 ‰. (Figs. 1d, 2d)

At temperature 35 °C (Fig. 1 e), maximum average value of survivals was 4.3 ± 1.5 individuals (21.66 %) at 55 ‰ and the minimum was 1.6 ± 0.5 individuals (8.3 %) attained at 115 ‰. The highest average growth was 4.938 ± 0.07 mm at salinity 115 ‰ and the lowest was 4.04 ± 0.08 mm at 75 ‰. Biomass exhibits a peak value of 26.87 ± 12.87 mg DW/L at 55 ‰ while the lowest value was 12.60 ± 4.69 mg DW/L at 115 ‰ (Fig. 2 d).

The salinity-temperature effect emphasized significant difference ($p<0.05$) in the survivals at 35 °C between salinities of 55 and 115 ‰ only. Whereas, the growth at this temperature differs significantly at 35 ‰ from 55 ‰, 95 ‰ and 115 ‰, and differs so at 55 ‰ from the other salinity concentrations. The biomass, on the other hand, did not indicate significant differences at the various salinities tested (Figs. 1 e, 2e).

Discussion

Temperature and salinity are considered as chief factors affecting the biology and distribution of aquatic invertebrates (Kinne, 1963), and temperature is often reported as most important than salinity, it interacts with salinity and each affecting the tolerance ranges of the other (Browne and Wanigasekera, 2000).

The present results clearly emphasize the combined effects of temperature and salinity on the larvae of local *A. franciscana*, so that the highest mean values of survivals of the larvae throughout the duration of the experiment (14 days) were at 20 °C and 55 ‰ and the lowest survivals were at 35 °C and 115 ‰. Obviously, the present results indicate that the rise in temperature caused considerably higher mortalities and this is in support of the result of Karim (1974) who achieved 100 % survival of nauplii of *Artemia* in India at temperature range of 5-20 °C, over 24h, however, this value declined greatly (18.3 %) at 30 °C, and at 36 °C all the larvae were died. Similarly, Vos and Tansutapanit (1979) showed that a Brazilian strain of *Artemia* from Macau survived for weeks at temperature around 40 °C. However, Vanhaecke *et al.* (1984), studied the responses of 13 different strains of *Artemia* to the combined effect of temperature and salinity and found that all strains, except that of Chaplin Lake (Canada) showed high survival over a wide range of salinities (35-110 ‰), and for all strains the common optimum temperature was between 20-25 °C. Moreover, Saygi (2004) pointed out that although survivals of two parthenogenetic strains of *Artemia* (one from Camalti, Izmer, Turkey and the other from Kalloni, Lebos, Greece), were significantly different from each other at 25 and 30 °C, neither of the strains exhibited good performance at high temperatures and the optimum salinity of the Camalti population was 80 ‰, and that of the Kalloni population was 120 ‰.

Strains of *Artemia* from Basrah undergoes 100 % mortality at 30 °C in 9 days (Ahmed, 2002), and a better survival (93 %) was obtained at 25 °C (Al-Obaydi, 2005). These two studies together with the present one recorded substantial mortalities at 30-35 °C, and they all agree that survival was best at 20-25 °C. This is in contrast with the conclusion drawn by Persoone and Sorgeloos (1980) that the highest temperature tolerance of a given *Artemia* strain was approaching 35 °C, and concur with that of Vanhaecke *et al.*(1984) that the optimum range of temperature in which survival of *Artemia* larvae is best was 20-25 °C.

The present results indicate a fairly good growth of larvae of local strain of *A. franciscana*, during 2 weeks at 30 °C, whereas, a decline in growth was apparent at 10 °C. This is in support with the result of Reeve (1963) on larvae of a strain of *Artemia* from the Great Salt Lake in Utah, USA, as the best growth was at 30 °C and 35 ‰, while the least growth was at 10 °C and 35 ‰. Moreover, Ahmed and Salman (1999) showed that growth of larvae and preadult *Artemia* increased with rise of temperature and decline of salinity below 70 ‰. Furthermore, the average growth of *Artemia* was significantly increased with the increase of temperature to 15-30 °C (Ahmed, 2002). However, it is important to emphasize that the last two studies were done on strain of *Artemia* collected from localities close to each other and about 12 km of the site from which the present specimens were collected. It is apparent, therefore, that all these bisexual strains are belonging to the same species (see Salman *et al.*, 2012), yet the populations may be different. Moreover, both temperature and salinity are major determinants of survivals, growth and reproduction in *Artemia franciscana* despite it's euryhalinity and eurythermity which may show phenotypic variation according to location (Irwin *et al.*, 2007). Moreover, natural selection compares heritable variants for their effect on reproduction, therefore, difference between populations from various localities are interpreted as the result of adaptation to different environmental conditions (Gajardo *et al.*, 2001).

The present results show that, with the exception of 20 °C, the highest average growth, occurred at the lowest level of survivals at temperature 30 and 35 °C. This emphasize that as the number of larvae declined, the average growth increases hence inversed correlation was obtained between density and growth (r= -0.684).

This suggests that as the number of larvae decreased the competition for food decreased. This coincides with the results of Evjemo and Olsen (1999), and that of Al-Obaydi (2005), however, the density in the last study was 50-400 larvae/L, whereas that of the present one was 1000 larvae/L.

Among other factors affecting survivals and growth are food quality and dissolved oxygen which interacts with each other and with salinity and temperature. Food is a limiting factor for survivals and growth, so as dissolved oxygen as the latter is inversely correlated with salinity and temperature, therefore, decline of oxygen concentration negatively affects survival and growth (Barata *et al.*, 1996, Varo *et al.*, 1993). It is worth mentioning that continuous aeration was not used here, as it severely affects the larvae and leads to great decline in average growth (Nimura, 1967).

One of other crucial factors is illumination during feeding the larvae; Sorgeloos (1972) suggests feeding larvae in darkness, because the larvae are photopositive and this would drive the larvae to concentrate in one place, and increasing competition for food, therefore, the energy spent in searching for food is more, which ultimately reduces growth and survivals.

The present results showed that the best survivals was at 55 ‰ and the least was at 115 ‰, this may be due to the fact that *A. franciscana* prefers lower salinities as the majority of species or strains of *Artemia* of the World prefer lower salinities to utilize most of the energy for survival, growth and reproduction and hence channeling less energy for osmoregulation (Ahmed, 1999; Persoone and Sorgeloos, 1980,), although having the most efficient system of osmoregulation ever exist in the animal kingdom (Crogham, 1958), this provides good adaptation to live in habitats devoid of predators (Van Stappen, 2002), nevertheless, growth was insignificantly different ($p>0.05$) at the various salinities used. However, higher survival of *Artemia* sp. nauplii from India was observed in seawater (80 %) (Soundarapandian and Saravanakumar, 2009).

It is evident from the present results that the highest biomass achieved was at 20 °C and 55 ‰, this may be considered as the optimum condition of temperature-salinity interaction for survival, growth and biomass production of *Artemia* larvae. Therefore, determination of optimum condition to achieve best growth and biomass is considered as a priority for mass culture of *Artemia* to be used in fish hatcheries and for economic consideration. This is concur with the results of Dhont and Lavens (1996) that the best average survivals, food assimilation and best biomass production in *Artemia*, at a controlled culture condition, were achieved at temperature 19-25 °C and salinity 32-65

In conclusion, the present investigation indicates that best survivals and best biomass of the local strain of *A. franciscana* from Basrah were achieved at temperature 20 °C and salinity 55 ‰.

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تأثير درجة الحرارة والملوحة على نسبة البقاء والنمو في الارتيميا *Artemia franciscana* من البصرة، العراق

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الخلاصة

اجريت التجارب المختبرية لدراسة تأثير درجة الحرارة والملوحة اضافة لتأثيرهما المشترك على البقاء والنمو والكتلة الحيه للنوع ارتيميا فرانسسكانا في مديات حرارة 10، 20، 25، 30 و35 م°، وملوحة 35، 55، 75، 95 و 115 ‰. كانت افضل نسبة بقاء هي(93%) في بدرجة حرارة 20 م°، ونمو (4.75) ملم وكتلة حية 122.8 ملغم وزن جاف/ لتر، بينما كانت ادنى فرصة بقاء (21%) في درجة حرارة 35 م°. كانت ادنى نسبة نمو وكتلة حية في درجة حرارة 10 م° حيث بلغت 1.66 ملم و 13.16 ملغم وزن جاف/ لتر. افضل نسبة بقاء رافقت ملوحة 55 ‰ وكانت 93%، بينما اعلى كتلة حية 87.122 ملغم وزن جاف/لتر. كذلك كان النمو مرتفعا 4.94 ملم في ملوحة 95‰. اما التاثير المشترك للحرارة والملوحة فكان على درجة حرارة 20 م° وملوحة 55 ‰ حيث بلغت نسبة البقاء (93.33) وادنى معدل بقاء (8.3%) كان في حرارة 35 مئوية وملوحة 115 ‰. كان اعلى معدل نمو (6.1 ملم) في درجة حرارة 30 م° وملوحة 115 جزء بالالف، وادنى نمو (1.49 ملم) في درجة حرارة 10 م° وملوحة 95 ‰. كان معدل الكتله الحية (122.86 ملغم وزن جاف باللتر) في درجة حرارة 20 م° وملوحة 55 ‰ بينما ادنى معدل كتلة حية (3.79 ملغم وزن جاف باللتر) في درجة حرارة 10 م° وملوحة 115 ‰.

كلمات مفتاحية: البقاء، النمو، الكتلة الحية، *Artemia franciscana*، درجة الحرارة، الملوحة